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Table of Contents


Page

1. Staphylococcus Enterotoxin - M. S. Bergdoll, Food Research Institute, The University of Chicago, Chicago, Illinois . . . . . 1
2. Iron-Porphyrins and Oxidation-Reductions - E. S. Guzman Barron, The University of Chicago, Chicago, Illinois . . . . . 5
3. Radiation Sterilization of Foods - Robert G. Tischer, Quartermaster Food & Container Institute for the Armed Forces, Chicago, Illinois . . . . . 18
4. Meat in Special Diets - Franklin C. Bing, Consultant, Chicago, Illinois . . . . . 23
5. Recent Developments in Nutrition Research - Charles Glen King, The Nutrition Foundation, Inc., New York, New York . . . . . 30
6. Diet in Relation to Heart Disease - Frederick J. Stare, Harvard University, Cambridge, Massachusetts . . . . . 36
7. The Nutritive Value of Gelatin - A. E. Harper and C. A. Elvehjem, University of Wisconsin, Madison, Wisconsin . . . . . 40
8. Nutritional Requirements of Swine - R. W. Luecke, Michigan State College, East Lansing, Michigan . . . . . 49
9. Use of Animal Fats in Feeds - B. S. Schweigert, American Meat Institute Foundation, The University of Chicago, Chicago, Illinois . . . . . 55
10. Turbo Chill in the Meat Industry - Harold F. Hagen, Union Stock Yard and Transit Company of Chicago, Chicago, Illinois . . . . 59
11. Livestock and Meat Research - T. C. Byerly, Animal and Poultry Husbandry Research Branch, U. S. Department of Agriculture, Washington, D.C. . . . . 68
12. Some Recent Research Which Concerns the Economic Aspect of Hides - Fred O'Flaherty, Tanners' Council Research Laboratory, University of Cincinnati, Cincinnati, Ohio . . . . . 78
13. Diathermal Processing of Meat - L. J. Pircon, American Meat Institute Foundation, The University of Chicago, Chicago, Illinois . . . . . 84

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I

# STAPHYLOCOCCUS ENTEROTOXIN

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Staphylococcus enterotoxin, the cause of staphylococcus food poisoning, has developed in a variety of foods. In processing foods to improve the quality, sometimes the improved products are more vulnerable to the growth of staphylococci. The only method that is sufficiently sensitive to determine the presence of toxin in foods implicated in food poisoning outbreaks is the feeding of human volunteers. Since human volunteers usually are not available, other methods of determining the presence of enterotoxin must be used. These methods are indirect and are hampered by the lack of satisfactory assay procedures. A few years ago Dr. Dack, Director of the Food Research Institute, with the help of a group of far-seeing men in the food industries initiated a project to develop a satisfactory assay method for enterotoxin. Since very little was known about the chemical nature of enterotoxin the initial goal was to obtain the enterotoxin in the pure state and determine its chemical properties. This information was to be used in developing a good chemical test for enterotoxin.

As the purification studies progressed it became apparent that enterotoxin was produced in very small quantities in culture media. In fact the amounts were so small that in order to obtain enough enterotoxin to continue the isolation studies it became necessary to move production to the pilot plant. At this point it seemed unlikely that a specific chemical test could be developed that would be sufficiently sensitive to detect toxin in foods. It appeared that if a practical test were to be developed it would most likely be done with information obtained from two other phases of the investigation, namely, immunological studies and pharmacological and biochemical studies. This change in view did not necessitate any change in the initial goal to isolate entertoxin because the ultimate success of the other studies depends on the availability of pure toxin.

At the present time we have a well integrated research program in progress with Dr. Surgalla in charge of the production and immunology studies, Dr. Sugiyama in charge of pharmacological and biochemical studies, and Dr. Bergdoll in charge of the chemical studies. Such an undertaking has been fruitful because of the participation and continued support and cooperation of the food industries.

Since it is impossible to cover all the phases of the work in the discussion I am going to confine my remarks to the chemistry of enterotoxin. It must be made clear that the following remarks are not about pure enterotoxin, but about a mixture of substances one of which is enterotoxin.

Evidence accumulated during this study indicates that enterotoxin is a protein or a derivative thereof. Some of the investigators in this field, have concluded that enterotoxin is a large, complex carbohydrate, and is not a protein. This difference of opinion is understandable when we consider that most enterotoxin preparations that have been obtained probably contained less

than one per cent toxin. We feel that the investigators contending that enterotoxin is a carbohydrate drew their conclusions from the properties of samples that contained much smaller percentages of enterotoxin that the preparations studied by those holding the view that enterotoxin was a protein. Evidence in this controversy may be secured from estimations of the yields of enterotoxic materials obtained by the investigators and the approximate dosage by weight required to cause illness in the test animals. For example, Hammon (1) who concluded that enterotoxin was a polysaccharide obtained approximately 720 mg of enterotoxic material per liter of crude bacterial filtrate. The amount required to cause illness in kittens was not less than 3.6 mg per animal. The most potent samples that we have obtained in the dry state gave positive reactions in monkeys when 0.03 mg was fed to each animal. If we estimate that a kitten is 25 times as sensitive by injection to enterotoxin as the monkey is by mouth, it would have required only 0.001 mg of our material to obtain positive reactions in kittens. Approximately 2 mg of toxic material of this potency was obtained from each liter of crude bacterial filtrate. We have estimated that these preparations contained not more than ten per cent enterotoxin by weight, which would indicate that Hammon's samples contained less than 0.01 per cent enterotoxin.

Investigators, concluding that enterotoxin was a carbohydrate, used similar methods in the preparation of their samples. With these methods one might expect to obtain high molecular weight carbohydrates as well as some proteins. The investigators who concluded that enterotoxin was a protein prepared their toxin samples by methods that have been employed to purify proteins. The latter preparations were several hundred times as potent as materials obtained by the other methods.

In our attempts to purify enterotoxin we have found many of the methods that have been employed in the isolation of proteins to be useful. Precipitation of enterotoxin with ammonium sulfate can be accomplished with a range of concentrations of this salt. Saturated sulfate solutions have been used most frequently, however, because precipitation of the enterotoxin from other concentrations is incomplete (2).

Much of the enterotoxin can be recovered from bacterial filtrates by adjustment of the pH to 3.5 followed by filtration with the aid of filter aids such as Hyflo Super-Cel. This is the most satisfactory procedure that we have found for recovering the enterotoxin from large volumes of crude filtrates.

Precipitation of enterotoxin with 40 per cent ethanol can be accomplished at pH 6.0 at -7° C. The inadequacy of our assay method renders it impossible to evaluate the degree of purification of enterotoxin by this procedure. This alcohol precipitation method of Cohn (3) has been used to isolate many proteins that could not be obtained by other methods.

Enterotoxin can be precipitated with zinc, but the difficulties involved in removing the zinc from the precipitates have led us to look for simpler methods of purification.

Chromatography, which has been very useful in the separation of many chemical compounds, has not been extensively employed in the purification of proteins because many of the proteins are unstable. However, some of the milder adsorbents such as diatomaceous silica and the cation exchange resin, Amberlite IRC-50, have been used for the purification of a variety of protein type compounds. We have found that these two adsorbents as well as alumina can be used as aids in the purification of enterotoxin. More than ninety per cent of the contaminating material in partially purified toxin preparations can be eliminated by chromatographing these materials on diatomaceous silica or alumina (4). The procedure with alumina, as the adsorbent has been adapted for use with the acid, precipitates from 600 liter lots of bacterial filtrates.

Many attempts have been made to chromatograph enterotoxin on Amberlite IRC-50, but only recently have we been able to obtain consistent results. Experiments are now in progress to find the optimum conditions for purifying the toxin with this adsorbent.

A number of proteins have been purified by electrophoresis with paper or starch as the supporting medium. We have been only partially successful in our attempts to apply these procedures to the purification of enterotoxin. Some encouraging results have been obtained with starch electrophoresis, but not enough evidence has been gathered to predict how much purification can be accomplished with this procudure.

At the present time partially purified enterotoxin preparations are obtained from 160 gallon lots of crude bacterial filtrate by the following series of procedures: (1) Precipitation at pH 3.5 followed by filtration through Hyflo Super-Cel, (2) adsorption on alumina followed by elution with phosphate buffer, (3) precipitation from 40 per cent ethanol at -7° C., (4) dialysis, and (5) lyophilization. The yield is 5-10 mg of dry sample per liter of bacterial filtrate. Usually 1 mg per monkey of this material will give positive reactions. Although these preparations are mixtures one might expect the components to be somewhat similar in chemical nature, since a series of procedures was used to obtain these mixtures. If this is true then the chemical properties of this material might indicate the general chemical nature of enterotoxin.

The carbohydrate content of these enterotoxin preparations was shown to be five per cent or less by both the Molisch and anthrone color tests. This does not exclude the possible presence of glycoproteins since many of them contain as little as five per cent carbohydrate.

The nitrogen in these preparations, which varied from 6 to 14 per cent, was indicated to be present in the form of proteins by both the Biuret test and Lowry's modification of the Folin method (5). Qualitative analysis by paper chromatography indicated that these proteins were composed of at least 17 amino acids. Semi-quantitative analysis with ion-exchange columns showed that aspartic acid, glutamic acid, lysine, and alanine may account for 60 per cent of the amino acid content of the material. Glycine, proline, and the leucines were present in greater amounts than serine, valine, threonine, tyrosine and phenylalanine. Only very small amounts of arginine, histidine, methionine, and cystine were indicated.

Our first experiments with trypsin indicated that crude preparations of this enzyme inactivated enterotoxin, however, later experiments with crystalline trypsin showed no inactivation of the toxin. It appeared that trypsin had very little effect on any of the components in the partially purified toxic preparations.

The molecular weight of enterotoxin is probably between 3000 and 50,000. This is indicated by the fact that enterotoxin will not pass through a dialyzing bag, but will pass through an ultrafilter that will barely pass albumin.

Evidence has been presented by several investigators that enterotoxin is relatively heat stable. Boiling crude filtrates for thirty minutes before feeding tham to human volunteers or injecting them intravenously into cats and monkeys did not seem to affect the potency of the toxin. However, Dolman (6) stated in one of his papers that the toxin produced by some strains of staphylococcus did seem to be more heat labile than that produced by other strains. During the present investigation results have been obtained with oral injections of monkeys with partially purified enterotoxin indicating that enterotoxin is progressively destroyed by heat, although some activity may remain after boiling for 30 minutes. Probably as much as 95% of the enterotoxin present in partially purified preparations was inactivated when solutions of these materials were held at 100° C. for thirty minutes at pH 6.0 and 7.5.

More could be said about the chemistry of enterotoxin, but I believe this

discussion gives you a resumé of our present knowledge of the subject. Definite progress toward the goal of obtaining pure enterotoxin is being made.

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II

# IRON-PORPHYRINS AND OXIDATION-REDUCTIONS

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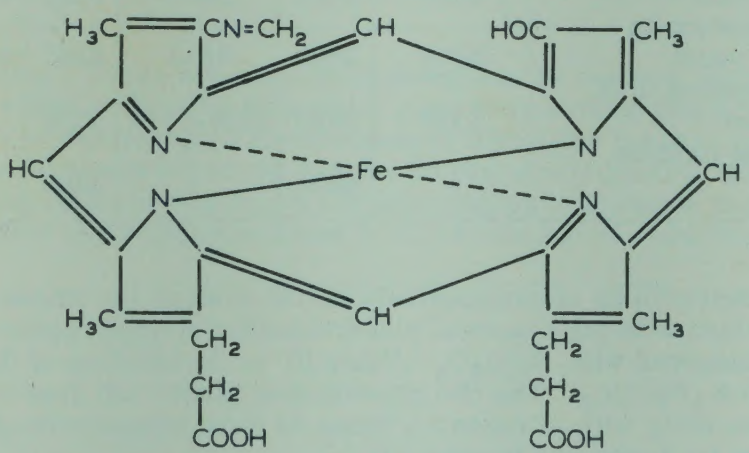
No other pigment in living cells has attracted so much attention as the iron-porphyrins. No wonder, as they are the respiratory agents in the truest sense, either transporting and storing oxygen or reacting with it through exchange of electrons. From the evolutionary point of view they seem to have made their appearance later than the other catalysts for biological oxidation-reductions (pyridine nucleotides, flavoproteins) because life appeared on earth before atmospheric oxygen, if we accept Oparin's concept of the origin of life (1). The first porphyrin pigments to appear must have been the Mg-porphyrin, such as those found in chlorophyll. Photosynthesis brought oxygen into the atmosphere and made respiration possible. The porphyrin of chlorophyll became transformed into the porphyrin of cytochrome oxidase. Warburg (2, 3) has suggested that the porphyrin nucleus of this pigment contains the formyl group of the porphyrin of chlorophyll:




Chlorocruorin resembles more closely cytochrome oxidase. It must have appeared before hemoglobin. The cytochromes, myoglobin and hemoglobin must have made their appearance in this order, the latter two only when, in the organization of polycellular organisms, the "milieu interieur" of Claude Bernard became established. A pigment similar to chlorocruorin can be prepared by addition of pheohemin to the protein of chlorocruorin. (See, however, Fox (4)).

The prophyrin molecule can easily accept metals. Thus iron enters into the center of the square formed by the four nitrogen atoms in the porphyrin ring stabilizing the electrons of the pyrrol nuclei. As a consequence, the absorption spectrum of the porphyrin loses some of its complexity: two absorption maxima in the visible light vanish, and fluorescence disappears (Table I). This iron completes its Werner coordination complex by ligating to itself two more groups, one above and the other below the plane of the porphyrin molecule. In aqueous buffered solutions coordination may be completed with water, or with anions present in the solution.

Table 1 - Absorption Spectrum Bands of Some Porphyrins and Iron-Porphyrin Compounds

| Compound | Absorption bands | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| | Å | Å | Å | Å | Å |
| Etioporphyrin III | 6267 | 5665 | 5264 | 4944 | 4000 |
| Protoporphyrin | 6296-6048 | 5786 | 5353 | 5010 | 4000 |
| $Fe^{+++}$ - protoporphyrin (ferrihemin) | 6100 | - | - | 4925 | 3850 |
| $Fe^{++}$ - protoporphyrin (ferrohemin) | - | 5660 | 5370 | - | 4100 |
| Cyanide ferriproto-porphyrin | - | 5450 | - | - | 4255 |
| Cyanide ferroproto-porphyrin | - | 5660 | 5370 | - | 4330 |
| Spirographisporphyrin | 6430-5925 | 5810 | 5550 | 5145 | ? |
| Cyanide ferrispirographis-porphyrin | - | - | 5500 | - | 4290 |
| Cyanide ferrospirographis-porphyrin | - | 5950 | 5400 | - | 4480 |
| Cytochrome c (ferro) | - | - | 5500 | 5200 | 4150 |
| Cytochrome system in bakers' yeast | 6030 | 5650 | 5500 | 5230 | 4490, 4330, 4170 |
| Cytochrome system in brewers' yeast | 5860 | 5650 | 5510 | 5250 | 4490, 4330, 4170 |
| Cytochrome system in B. azotobacter | 6320 | 5890 | 5630-5510 | 5260 | 4300 |
| Co-cytochrome oxidase, heart | - | 5890 | - | 5100 | 4500 |

Iron-porphyrin is an electromotively active system (5) whose potential can be determined with the classical potentiometric methods either by titration of the ferri-compound with $Na_2S_2O_4$ (Table II) or by titration of the ferro-compound with $K_3Fe(CN)_6$. The two coordination places left free combine easily and reversibly with nitrogenous bases to form hemochromogens, as was discovered by Anson and Mirsky (6):

$$Fe^{+++}\text{ porphyrin} + 2N \rightleftharpoons Fe^{+++}\text{ porphyrin} - N_2 \quad ;$$
$$Fe^{++}\text{ porphyrin} + 2N \rightleftharpoons Fe^{++}\text{ porphyrin} - N_2 \quad .$$

The degree of association, as determined by the value of $K_{ox}$ and $K_{red}$

$$K_{ox} = \frac{(Fe^{+++}\text{ porphyrin} - N_2)}{(Fe^{+++}\text{ porphyrin})\,(N)^2}$$

$$K_{red} = \frac{(Fe^{++}\text{ porphyrin} - N_2)}{(Fe^{++}\text{ porphyrin})\,(N)^2}$$

will determine the change of potential of iron-porphyrin on its conversion to

Table 2 - Titration of Ferrihemin with $Na_2S_2O_4$ in 0.1 N NaOH

Concentration of ferrihemin, 0.0005 M per liter, 75 cc. in titration vessel. t. 30°. $E_h = E'_o - 0.0601 \times \log (Fe^{++}$ hemin) / ($Fe^{+++}$ hemin). $E'_o = -0.4617$ volt (average figure of all titration values).

| Reducing agent $Na_2S_2O_4$ | Reduction | $E_h$ found | $E_h$ calculated | Difference |
|---|---|---|---|---|
| cc. | per cent | volt | volt | volt |
| 0.070 | 4.86 | -0.3830 | -0.3839 | -0.0009 |
| 0.128 | 8.89 | -0.4013 | -0.4019 | -0.0006 |
| 0.225 | 15.62 | -0.4143 | -0.4173 | -0.0030 |
| 0.305 | 21.18 | -0.4243 | -0.4274 | -0.0031 |
| 0.398 | 27.63 | -0.4337 | -0.4365 | -0.0028 |
| 0.480 | 33.32 | -0.4420 | -0.4436 | -0.0016 |
| 0.565 | 39.23 | -0.4500 | -0.4503 | -0.0003 |
| 0.666 | 46.25 | -0.4582 | -0.4578 | +0.0004 |
| 0.771 | 53.58 | -0.4669 | -0.4655 | +0.0014 |
| 0.890 | 61.80 | -0.4762 | -0.4743 | +0.0019 |
| 0.991 | 68.1 | -0.4846 | -0.4815 | +0.0031 |
| 1.090 | 75.7 | -0.4930 | -0.4914 | +0.0016 |
| 1.210 | 84.1 | -0.5070 | -0.5052 | +0.0018 |
| 1.330 | 92.4 | -0.5265 | -0.5270 | -0.0014 |
| 1.410 | 97.9 | -0.5650 | -0.5622 | +0.0028 |

hemochromogen. There are also other complicating factors, such as dimerization (7) and effect of $H^+$ —factors which have been studied quantitatively by Clark and his students (8). Hemochromogen formation leads to greater stability of the electronic structure of the system so that electrometric titration is made easier. By the addition of a variety of nitrogenous compounds there thus are created a series of hemochromogens of varied oxidation-reduction potentials (Fig. 1).

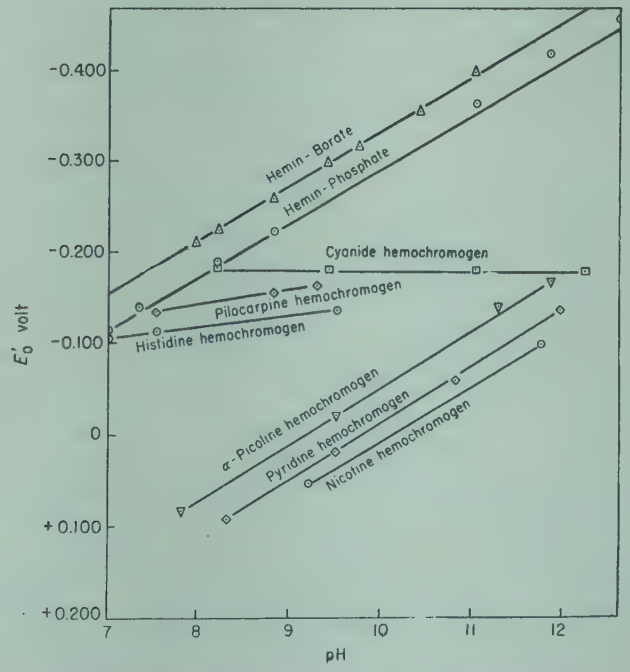



Figure 1. The relation pf pH values to the $E'_o$ values of iron-protoporphyrin (hemin) and its hemochromogens.

The change of potential of iron-porphyrins on addition of nitrogenous compounds leading to the formation of systems of different free-energies (Table III) is of great importance to their catalytic power. As the rate of

Table 3 - Oxidation-Reduction Potentials of Some Iron-Porphyrins and Their Nitrogenous Derivatives

| System (Temp. 30°) | pH | $E'_o$ in volts |
|---|---|---|
| Fe-protoporphyrin | 9.63 | -0.316 |
| CN-Fe-protoporphyrin | " | -0.183 |
| Fe-spirographisporphyrin | " | -0.230 |
| CN-Fe-spirographisporphyrin | " | -0.113 |
| CN-Fe-hematoporphyrin | " | -0.200 |
| CN-Fe-mesoporphyrin | " | -0.229 |
| CN-Fe-coproporphyrin | " | -0.247 |
| Picoline Fe-spirographisporphyrin | " | -0.010 |
| Picoline Fe-protoporphyrin | " | -0.033 |
| Picoline Fe-hematoporphyrin | " | -0.099 |
| Cytochrome b | 7 | -0.04 |
| Cytochrome c | " | +0.255 |
| Cytochrome a | " | +0.29 |
| Hemoglobin | " | +0.135 |
| Hemoglobin in urea | " | +0.106 |
| Myoglobin | " | +0.046 |

oxidation of electroactive dyes by atmospheric oxygen is a function of the free-energy of the system (9), so the catalytic power of hemochromogens depends on the oxidation-reduction potential of the system. This relationship can be seen in the oxidation of ascorbic acid (Fig. 2) and in the oxidation of lactate activated by lactic dehydrogenase (Fig. 3). The oxidation of unsaturated fatty acids is also catalyzed by iron-porphyrins, although here the iron-porphyrin seems only to initiate a chain reaction, which once started proceeds without the aid of the catalyst (Fig. 4). All these hemochromogens owe their catalytic power to their property of being electroactive and readily oxidized by atmospheric oxygen.

In the living cell these hemochromogens or cytochromes are formed by combination with proteins, a combination which is more firm than that of the hemochromogens produced with nitrogenous bases. In cytochrome c, for example, the iron-porphyrin disk is firmly fixed from both sides by the hemochromogen-forming groups of the protein, the histidine residues (10). In this way the iron is protected, and oxidation with oxygen does not take place. Cytochrome c is thus a sluggish system which is oxidized or reduced in the cell by electro-active systems (flavoproteins, cytochrome oxidase). At physiologic pH values it does not combine with cyanide or with CO. In strongly acid or alkaline solutions there is a break of one of the iron protein bonds, and combination with cyanide and CO becomes possible. The protein molecule is remarkably stable, for it resists the action of such strong acids as $CHCl_3COOH$. Little is known about the other cytochromes, except perhaps cytochrome b, which seems to be a component of lactic dehydrogenase of yeast (11, 12) and perhaps of succinic dehydrogenase. That various cytochromes in the b and the a group have been reported is strong indication of the existence of several specific proteins which on combination

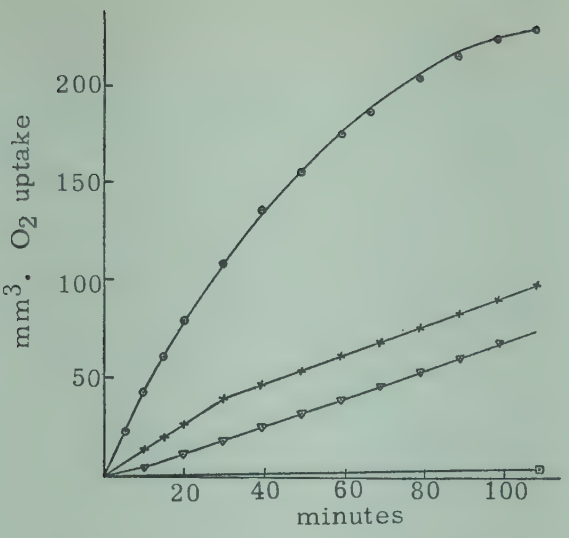



Figure 2. The oxidation of ascorbic acid by atmospheric oxygen with hemochromogens as catalysts. pH 6.39. Temperature, 25° C. Amount of ascorbic acid, 0.02 mM. Amount of hemin in the hemochromogens, 0.00015 mM. Curve 1, control; curve 2, pilocarpine hemochromogen; curve 3, pyridine hemochromogen; curve 4, nicotine hemochromogen. Curves numbered from bottom (Curve 1) to top (Curve 4).

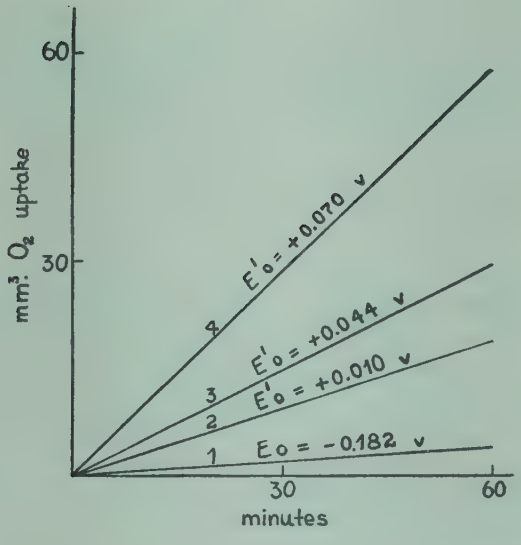



Figure 3. The oxidation of lactate by hemochromogens. pH, 9. Temp. 37°. Enzyme, lactic dehydrogenase from gonococci.

1. Cyanide hemochromogen;
2. Picoline hemochromogen;
3. Pyridine hemochromogen;
4. Nicotine hemochromogen (28)

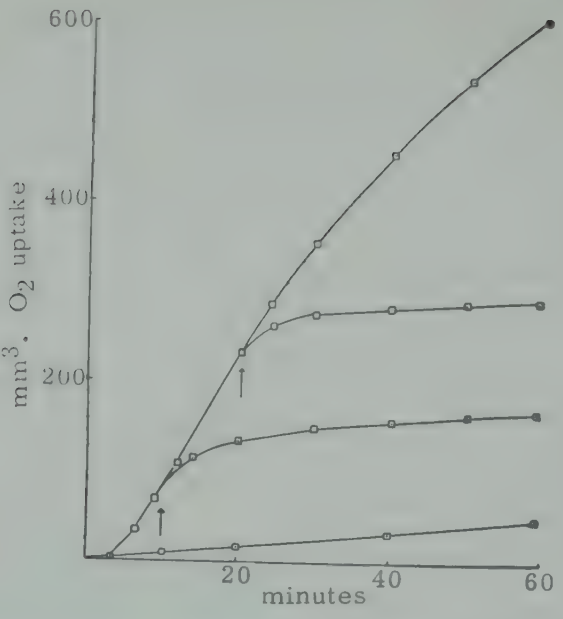



Figure 4. The effect of p-aminophenol (0.001 M) on the oxidation of linseed oil with hemin as catalyst. pH 6.96. Temperature, 28° C. Curve 1, control; curve 2, p-aminophenol added 20 minutes after hemin addition; curve 3, p-aminophenol added 10 minutes after hemin addition; curve 4, p-aminophenol added before hemin addition. Curves numbered from top (Curve 1) to bottom (Curve 4).

with iron-porphyrin give cytochromes differing from each other in their spectroscopic properties. In the aerobic cell they are the universal electron-transfer catalysts from flavoproteins to atmospheric oxygen, a transfer which proceeds from the system of more negative potential to the system of more positive potential:

$$XH + Fe^{++}\ \text{cyt.}\underline{b} \rightarrow Fe^{++}\ \text{cyt.}\underline{c} \rightarrow Fe^{++}\ \text{cyt.}\underline{a} \rightarrow Fe^{++}\ \text{cyt.oxidase} \longrightarrow O_2 - O_2^{\,-} + H^+$$

In every case oxidation-reduction takes place by loss or gain of one electron. Transfer of electrons from flavoprotein to cytochrome must therefore occur in two steps—the first cytochrome molecule oxidizing the flavin to the free radical half-oxidation product, and the second completing the reduction:

$$Fe^{+++}\ \text{cyt.}\underline{c} + FH_2 \rightarrow Fe^{++}\ \text{cyt.}\underline{c} + FH + H^+$$
$$Fe^{+++}\ \text{cyt.}\underline{c} + FH \rightarrow Fe^{++}\ \text{cyt.}\underline{c} + F + H^+$$

The mechanism of oxidation of Warburg's Sauerstoffübertragendes Ferment, cytochrome oxidase, is still unknown. It can be oxidized at very low pressures and there may well be a direct electron transfer between oxygen and iron:

$$Fe^{++} + O_2 \rightarrow Fe^{+++} + O_2^{-} \quad ;$$
$$O_2^{-} + H^{+} \rightarrow O_2H \quad ;$$
$$O_2H + Fe^{++} \rightarrow Fe^{+++} + HO_2^{-} \quad ;$$
$$HO_2^{-} + H^{+} \rightarrow H_2O_2 \quad .$$

Or it may first form a loose complex with oxygen similar to the blue intermediate complex of ferrous cysteine and oxygen.

In animal tissues molecular oxygen is transported to the tissues by the iron-porphyrin pigments hemoglobin, myoglobin and chlorocruorin, and by the copper-containing pigment hemocyanin.

The hemoglobin molecule is a flattened disk whose shape may be approximated by a right-circular cylinder 34 Å high and 57 Å in diameter. It possesses a twofold axis of symmetry, perpendicular to the cylinder axis, and appears to consist of four equally spaced layers of matter. The four iron-prophyrins are arranged on the surface of the molecule with their planes parallel to the cylinder axis and the axis of symmetry, and are attached to identical residues of the protein, so that inherently thay are all alike. They are grouped in two identical pairs and the molecule can be reversibly split in two (13). The specificity of the protein component is such that foetal and adult hemoglobins have proteins of different amino acid content and different oxygen affinities (14). In fact Itano (15) has demonstrated that the blood of the same individual may contain different kinds of hemoglobin. The globin component differs not only from species to species, but in the same species with age and other factors. The iron-porphyrin component is identical in all hemoglobins.

Hemoglobin has the remarkable property of combining reversibly with molecular oxygen without any change in the valency state of iron which remains in the ferrous state both in hemoglobin and in oxyhemoglobin. As we have already stated this property does not exist in cytochromes. Hemoglobin can also be oxidized to the brown ferri-pigment methemoglobin, a process which resembles that of oxidation-reduction among the cytochromes.

The mode of attachment of oxygen to the hemoglobin molecule is still unknown. It is known, however, that attachment takes place through the iron-porphyrin nucleus, since four oxygen molecules combine with one mole of hemoglobin. This is the reason for the S shaped curve of the oxygen dissociation in hemoglobin. When the hemoglobin molecule is split in two, as happens on dilution, there is a change in the oxygen dissociation curve. All the factors which control affinity of oxygen in the hemoglobin molecule are not yet known. Among them is the ionic environment. (Fig. 5). Oxygen saturation of hemoglobin varied with the salt dissolved in the aqueous solution (16).

Perhaps an indication that hemoglobin is different among different individuals within the same species is given by the half-saturation values for $O_2$. In the Chilean expedition of 1935, the blood hemoglobin of all the members of the expedition had different half-saturation values, both at sea level and at 20,000 feet (Table IV). At 17,000 feet, the hemoglobin of the residents had greater affinity for oxygen than the hemoglobin of the expedition members. Indentical findings were made on the blood of other animals and birds. In general half-saturation values for blood of mammals is between 26 and 43 mm Hg. The blood of the llama and the vicuña—both indigenous of the high Andean plateau—had greater affinity (Fig. 6). The blood of the South American ostrich, and of the huallata (a bird found only in the South American Andes above 14,000 feet) had also higher affinity for oxygen than the blood of other birds (Fig. 7). Undoubtedly the residents of high altitude have developed a hemoglobin with greater oxygen affinity than that of individuals living at sea level.

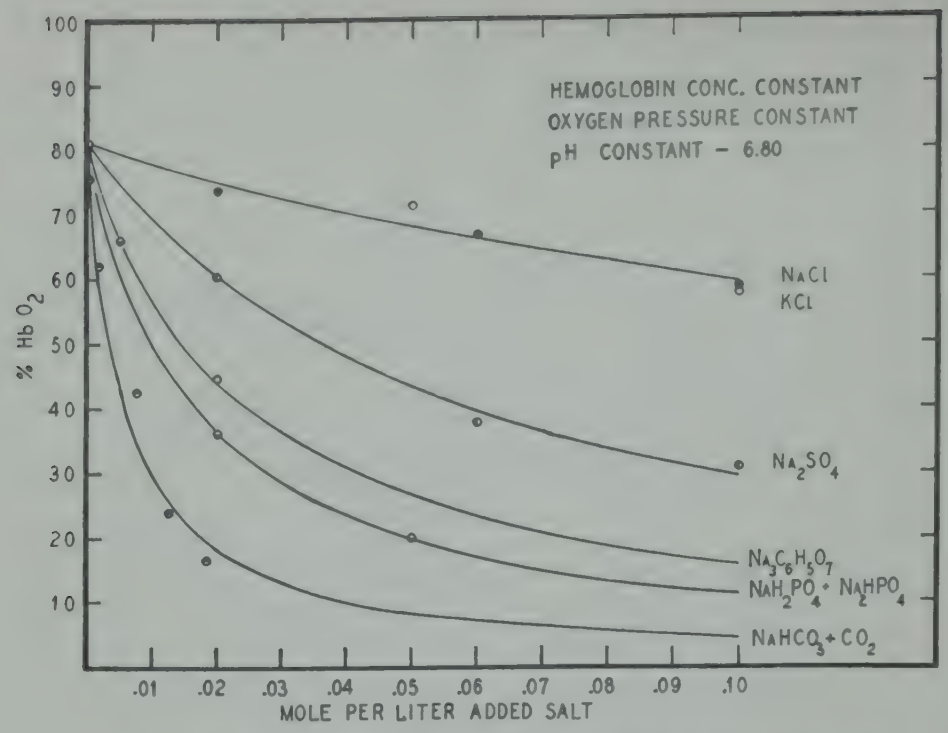



Figure 5. Saturation of Hemoglobin as a function of the salt concentration.

Another property which is common to both, Warburg's Sauerstoffüber-tragendes Ferment and hemoglobin, is the combination with CO, reversibly dissociated by light. This reaction, which takes place also in myoglobin and chlorocruorin, occurs only with the ferro form. In all cases studied the total combining power of the pigment for CO is, like that for $O_2$, equal to the number of iron-porphyrins in the molecule. When the pigment is exposed to a mixture of the two gases at sufficiently high pressure, combination with either gas can occur only at the expense of combination with the other. Affinity of CO for hemoglobin is very high, and the partition coefficient value a:

$$\frac{HbCO}{HbO_2} = \frac{a\ pCO}{pO_2}$$

ranges from 125 to 550 depending on the origin of hemoglobin. This value for vertebrate myoglobin is from 28 to 50, and for Warburg's respiration ferment, 0.1.

Hemoglobin can also be oxidized by a loss of one electron by the ferro-porphyrin to the brown ferri-porphyrin pigment, methemoglobin. This oxidation-reduction system belongs, like cytochrome c, to the group of sluggish oxidation-reduction systems. The potentials cannot therefore be measured by the classical potentiometric methods unless an electroactive mediator is added (19). It must be remembered that while cytochromes, cytochrome oxidase (Warburg's respiration ferment), and myoglobin have one iron-porphyrin per molecule of protein, hemoglobin in dilute solutions or in 4 M urea has two, and hemoglobin in concentrated solutions has four iron-porphyrins. The equation for the oxidation-reduction potentials,

$$Eh = Eo - \frac{RT}{nF} \ln \frac{(Red)}{(ox)}$$

Table 4 - Position of the Oxygen Dissociation Curve at Various Altitudes.

Values for $pO_2$ at half saturation are in mm. Hg and are at 37.0°C for contsant $pH_c$ = 7.10 and at the observed rectal temperature for the $pH_c$ of the arterial blood as drawn.

| Subject | At Sea Level (Boston) | | | | |
|---|---|---|---|---|---|
| | Days after arrival | Arterial $pH_c$ | $HbO_2$ capacity vols. per cent | $pO_2$ for Hb. = $HbO_2$ | |
| | | | | arter. $pH_c$ | $pH_c$ = 7.10 |
| Barron | 100 | 7.08 | 19.42 | 25.3 | 25.1 |
| Christensen | | 7.12 | 19.33 | 26.2 | 26.6 |
| Dill | | 7.10 | 18.22 | 26.8 | 26.9 |
| Edwards | | 7.10 | 19.62 | 25.5 | 25.7 |
| Forbes | | 7.15 | 20.37 | 25.3 | 26.1 |
| Hall | | 7.11 | 22.06 | 26.8 | 27.1 |
| Keys | | 7.11 | 18.60 | 26.9 | 27.2 |
| McFarland | 100 | 7.10 | 20.87 | 26.1 | 26.3 |
| Talbott | | 7.11 | 20.66 | 25.0 | 25.3 |
| Mean | | 7.11 | 19.91 | 25.98 | 26.28 |

| Subject | At 5.34 km., B. P. = 401 mm. (Quilcha) | | | | |
|---|---|---|---|---|---|
| | Days after arrival | Arterial $pH_c$ | $HbO_2$ capacity vols. per cent | $pO_2$ for Hb. = $HbO_2$ | |
| | | | | arter. $pH_c$ | $pH_c$ = 7.10 |
| Barron | 5 | 7.18 | 25.40 | 26.7 | 29.9 |
| Christensen | 4 | 7.71 | 23.70 | 26.9 | 29.7 |
| Dill | 4 | 7.12 | 22.94 | 29.1 | 30.1 |
| Edwards | 16 | 7.13 | 25.93 | 25.0 | 26.4 |
| Forbes | 5 | 7.13 | 23.73 | 27.1 | 28.4 |
| Hall | 1 | 7.14 | 25.55 | 28.3 | 30.2 |
| Keys | 3 | 7.17 | 23.72 | 26.6 | 29.5 |
| McFarland | 4 | 7.14 | 24.67 | 28.1 | 29.8 |
| Matthews | 4 | 7.12 | 23.70 | 25.8 | 27.0 |
| Talbott | 3 | 7.09 | 24.95 | 28.9 | 28.9 |
| Mean | | 7.14 | 24.43 | 27.3 | 28.99 |
| Alcaino | | 7.15 | 29.77 | 25.5 | 27.4 |
| Alcio | | 7.06 | 29.25 | 28.5 | 27.3 |
| Bastias | | 7.08 | 30.70 | 24.4 | 24.0 |
| Campos | | 7.07 | 32.72 | 27.5 | 26.6 |
| Fritz | | 7.11 | 24.62 | 27.2 | 27.6 |
| Heredia | | 7.06 | 34.20 | 29.0 | 27.8 |
| Martinez | | 7.13 | 19.87 | 25.8 | 27.1 |
| Troncoso | | | 32.54 | | 27.7 |
| Mean | | 7.09 | 29.21 | 26.8 | 26.94 |

Table 4 - continued.

| Subject | At 6.14 km., B.P. = 256 mm. (Punta) | | | | |
|---|---|---|---|---|---|
| | Days after arrival | Arterial $pH_c$ | $HbO_2$ capacity vols. per cent | $pO_2$ for Hb. = $HbO_2$ | |
| | | | | arter. $pH_c$ | $pH_c$ = 7.10 |
| Barron† | 2 hr. | 7.04 | 27.12 | 30.1 | 28.1 |
| Barron | 1 | 7.16 | 26.61 | 25.8 | 28.1 |
| Dill† | 2 hr. | | 22.59 | | 30.8 |
| Edwards† | 2 hr. | 7.07 | 25.37 | 28.5 | 27.6 |
| Forbes | 1 | 7.16 | 23.66 | 27.4 | 29.8 |
| Forbes § | 2 | 7.16 | 24.66 | 27.5 | 30.1 |
| Hall† | 2 hr. | 7.21 | 25.51 | 24.3 | 28.3 |
| Keys | 3 | | 24.38 | | 30.5 |
| Keys | 6 | 7.12 | 23.25 | 26.9 | 27.9 |
| McFarland | 2 | 7.18 | 26.33 | 27.5 | 30.7 |
| Matthews | 3 | 7.12 | 24.69 | 27.7 | 28.8 |
| Matthews | 6 | 7.11 | 24.42 | 28.1 | 28.8 |
| Talbott‡ | 1 | 7.07 | 25.27 | 30.1 | 29.2 |
| Mean | | 7.13 | 24.90 | 27.6 | 29.13 |

† After climbing from 5.43 km.
‡ Ill of acute mountain sickness.
§ A period of 2 days at 5.43 km. intervened before Forbes encamped at 6.14 km. for the second day.

has $n = 1$ in cytochromes and myoglobin; in urea-dissolved hemoglobin $n = 2$, while in hemoglobin in concentrated solution $n$ has different values; at half-oxidation it is close to 2; at a fractional oxidation less than half, $n$ drops to a considerably smaller value; and at fractional oxidations above half, $n$ rises over 2 (19, 20, 21).

Myoglobin, the tissue oxygen store, differs from hemoglobin by its smaller molecular weight (17,000 instead of 67,000), by its smaller number of iron-porphyrins per molecule (one instead of four), and by its greater affinity for oxygen (half-saturation, 1-2 mm instead of 25-27 mm). There must be as many different myoglobins as there are hemoglobins, and it is quite possible that the myoglobin of the high altitude dwellers differs from the myoglobin of the sea level individuals.

Oxidation of hemoglobin and myoglobin to methemoglobin or metmyoglobin is a problem of great importance to the meat industry because the brown color of meat is disliked by the consumer. Brooks (22) found that under constant conditions of oxygen pressure, pH, salt concentration, and temperature the oxidation of hemoglobin to methemoglobin by oxygen was a first order reaction with respect to the concentration of unoxidized hemoglobin and that the rate of oxidation had a well defined maximum value at a low oxygen pressure (about 20 mm at 30°). The oxidation of myoglobin at constant oxygen pressure, pH, salt concentration, and temperature is also first order with respect to unoxidized myoglobin (23, 24). The rate of oxidation has a clearly defined maximum at a partial pressure of about 1 mm. The rate diminishes up to 30 mm, and remains constant afterwards. The maximum rate of oxidation in both pigments is thus at the half-$O_2$

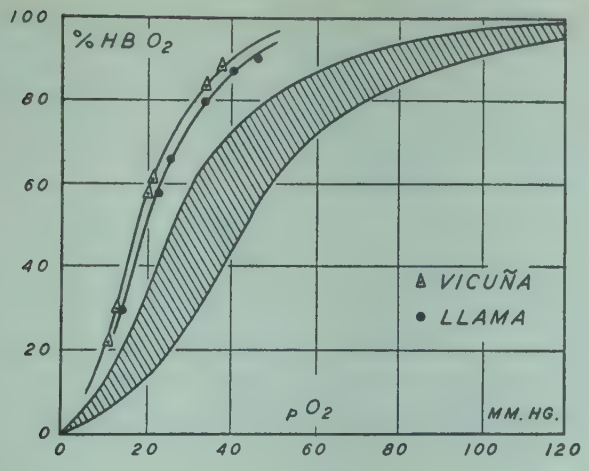



Figure 6. The oxygen dissociation curves of the llama and vicuña at body temperature (39°) and $pH_C = 7.1$ compared with the range for eight lowlanders. These eight range from man to sheep, the intermediate being the horse, dog, rabbit, pig, peccary and ox.
The viscacha, a native rodent of high altitudes, has an oxygen dissociation very near that of man.

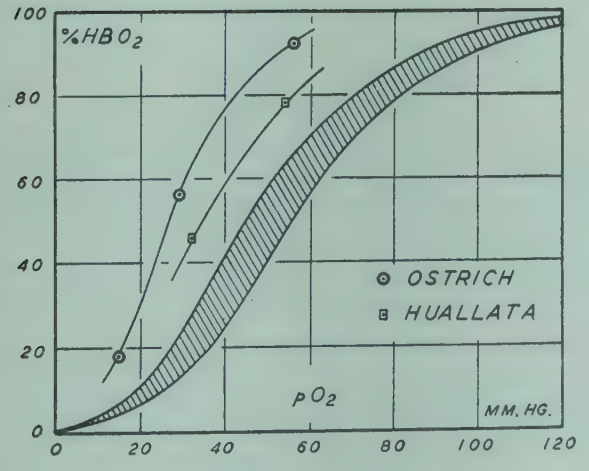



Figure 7. The oxygen dissociation curves of the two birds of high altitude compared with the range of six lowlanders. These range from the pigeon to the domestic fowl, the intermediate four being the muscovy duck, the domestic goose, the domestic duck and the Chinese pheasant.

saturation level. The activation energy for the oxidation of myoglobin at low oxygen pressures is about 19,000 calories, whereas at high oxygen pressures it rises to 25,000 calories. The presence of oxymyoglobin therefore hinders the reaction. The oxidation of myoglobin in air proceeds at a rate 4.25 times faster than the oxidation of hemoglobin, and the browing of meat must be due to the ready formation of metmyoglobin.

The simplest stoichiometry for the oxidation of ferrous compounds by atmospheric oxygen requires four $Fe^{++}$ equivalents per mole of $O_2$. In the oxidation of myoglobin, which has one $Fe^{++}$ porphyrin per mole of protein, George and Stratmann (23) found an uptake of 2.5 moles, an indication that some oxidizable groups of the proteins were oxidized during this process (tryosine, tryptophan, serine). During the oxidation of the $Fe^{++}$ compound there must be formation of intermediates which are strong oxidizing agents. In acid solutions the oxidation of oxyhemoglobin is first order with respect to oxyhemoglobin and hydrogen ions, the rate determining step involving the production of the perhydroxyl radical (24):

$$Fe^{++}HbO_2 + H^+ \rightarrow Fe^{+++}Hb + HO_2.$$

The faster oxidation of hemoglobin and myoglobin when compared to the oxidation of oxyhemoglobin and oxymyoglobin is an indication that there is formation of an auxiliary electron accepting group in the unoxidized forms of myoglobin and hemoglobin. In agreement with George and Stratmann (24) the following equations may be formulated to explain the oxidation of these two pigments:

$$Fe^{++}MbO_2 \underset{3}{\overset{1}{\rightleftharpoons}} Fe^{+++}Mb + O_2^-$$

$$Fe^{++}Mb + HO_2 \xrightarrow{2} Fe^{+++}Mb + O_2H^-$$

$$Fe^{++}MbO_2 + HO_2 \xrightarrow{2} Fe^{+++}Mb + O_2 + O_2H \text{ (slow)}$$

$$Fe^{++}Mb + O_2^- \xrightarrow{4} \overset{(red)}{MbFe^{++}} + O_2$$

$$Fe^{++}MbO_2 + O_2^- \xrightarrow{4} \overset{(red)}{MbO_2Fe^{++}} + O_2$$

$$\overset{(red)}{MbFe^{++}} + Fe^{+++}Mb \longrightarrow Fe^{++}Mb + Fe^{++}Mb$$

$$\overset{(red)}{MbO_2Fe^{++}}\, Fe^{+++}Mb \longrightarrow MbO_2Fe^{++} + Fe^{++}MbO_2$$

$$O_2H^- + H^+ \longrightarrow H_2O_2$$

$$2\,MbFe^{++} + H_2O_2 \longrightarrow 2Fe^{+++}Mb + 2OH^-,$$

where $Fe^{++}Mb$, $Fe^{++}MbO_2$, $Fe^{+++}Mb$, $\overset{(red)}{MbFe^{++}}$, and $\overset{(red)}{MbO_2Fe^{++}}$ are myoglobin, oxymyoglobin, metmyoglobin, the electron-accepting group of myoglobin and of oxymyoglobin, respectively.

There is great interest in the utilization of the killing power of ionizing radiations for the sterilization of foods. I believe this is a rather dangerous procedure, for ionizing radiations produce a large number of oxidations. For example x-rays oxidize rapidly ferro-porphyrin compounds such as ferro-cytochrome c (26) and hemoglobin (27). The oxidation of hemoglobin and of myoglobin to the ferri-compound is proportional to the x-ray dose and proceeds as a zero order reaction. With the x-ray or gamma ray doses ap-

plied to produce food sterilization, not only will all the hemoglobin be oxidized to methemoglobin, but there may also be further destruction of the porphyrin nucleus itself.

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III

# RADIATION STERILIZATION OF FOODS

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The discovery of a new tool, a new method or a new principle often sets off a wave of speculation about the impact of the new discovery on adjacent areas of interest. The appearance of atomic power on the scene in the Hiroshima incident engendered just such an effect—first with regard to the destructive effects which might be expected in full scale military use of atomic power and then, as further developments occurred and the latent possibilities of the new tool were brought into focus, in considering the broad spectrum of peace-time uses.

The possible use of atomic power for constructive purposes has roots which extend deep into the scientific literature. Radium and X-ray therapy are two outstanding examples of the use of atomic emanations which have been very fruitful.

The effect of radioactivity on biological systems has many facets, among them the field of food irradiation. Advances in food research have indicated that preservation of food by heat, dehydration, fermentation, addition of chemicals and biologicals most often produces a product possessing a set of characteristics radically different from those inherent in the fresh product.

As a result, the discovery of a method for preservation of food which would leave the quality of the fresh product virtually unaltered has been a constant motivation to food technologists. Sterilization of foods by irradiation with beta or gamma emanations appears to offer promise of attaining this goal.

The work of researchers in industry, academic institutions, the Atomic Energy Commission, and other agencies has currently developed food irradiation to a point where some of the highlights are clearly evident.

At present atomic power of high intensity is available from mechanical accelerators as well as from fission products. The future promises to provide more powerful accelerators as well as a continuous and growing source of fission products. Research adds daily to our understanding of the characteristics of this powerful tool.

In its present state of development, food research has indicated how much energy is necessary to attenuate or destroy types of microorganisms important in the preservation of food. It has also shown the possibility of eliminating insect infestation in certain cereal products. Enzymes have been found highly resistant to radioactivity and with most food products, the effect on odor, flavor, and color has been found to be destructive in one or another degree.

These facts were confirmed in a survey conducted by the Quartermaster Corps in April of 1953. A need for integration of efforts was noted and the decision was made to set up a program which would bring together scattered efforts and provide impetus for research aimed toward proving that preser-

vation of food by irradiation either is or is not a reasonable possibility.

The responsibility for conducting this program was placed with the Quartermaster Food & Container Institute, and in cooperation with the Atomic Energy Commission and researchers in this field, plans were made for carrying on the project.

Areas in need of attention were:

1. The cause of off-flavors and odors, to determine the nature of chemical changes in foods due to irradiation.

The opinion of workers in the field of food irradiation appears to be practically unanimous with regard to the effect of irradiation on the flavor of food. No case has been reported in which the flavor has been enhanced by these means, and in most instances off-flavors commensurate with the amount of irradiation applied have been noted. It is for this reason that studies of off-flavors and odors have been placed high on the list of priorities. In this complex situation, the most profitable approach appears to lie in the direction of determining the mechanism of changes in odor and flavor in the hope that knowledge of this mechanism will lead to the formulation of methods for the control or elimination of such negative effects.

From a technological point of view, it may be profitable to survey reactions of all of the important food items to the various levels of irradiation which it is now possible to use to determine whether there are some important commodities which will survive either pasteurization or sterilization processes without the accumulation of sufficient quality losses to make them unacceptable.

2. Fundamental research on the nature of volatile off-flavors and odors of irradiated food to provide means of identification and elimination.

A slightly different approach to the flavor problem, and certainly not a new one, lies in identifying the chemical constituents of flavors and odors produced by irradiation. Again, the objective is to use this information, if possible, in prescribing methods for the use of radiation processes which will eliminate off-odor and flavor.

3. Further work on the mechanism of destruction of insects.

Research to date on the destruction of insects has indicated that these organisms may be killed with relatively small amounts of irradiation. Considering the importance of ridding such commodities as stored cereal products of insect infestation without altering the useful properties of the product, it is clearly important to have further details concerning the application of irradiation to the various types of insects and commodities ordinarily involved.

4. Microbiological studies on food poisoning and spoilage organisms.

The microbiology of food spoilage is a well developed area of science which has been remarkably productive in allowing the production of a wide variety of satisfactory food products. As with any new process however, it will probably be necessary to ramify and extend our knowledge in this area for assurance that the concepts already established with regard to food commodities conventionally processed will hold also in the field of irradiation.

5. A comparison of the chemical effects of irradiation of foods by (a) cathode rays and X-rays and (b) fission products to determine whether one type possesses any singular advantages in food preservation.

Physicists, in general, appear to agree that the prospects of important differences in effect from the several radioactive emanations available for

processing are very unlikely. At the present time, however, there does not appear to be any complete evidence for or against this stand. This fact, coupled with the inherent characteristics of the machines or fission product sources dictate the necessity for some attention to this area.

6. Studies to determine the mechanism of enzymic changes in foods as affected by irradiation.

Work on enzyme systems has already given considerable indication that inactivation of many enzyme systems requires considerable more irradiation than is necessary, in general, to kill bacterial spores. Further work is needed to determine the effects of enzyme systems on quality.

7. A study of the effects of irradiation on the quality of pre-processed foods.

It may be possible to combine conventional processing techniques with irradiation to produce products possessing quality characteristics better than those which would be found in the product completely processed by either means alone. Considering the number of important foods and useful processes already available the testing of these combinations in differing proportions is one which may require considerable effort.

8. Toxicological studies designed to determine wholesomeness of irradiated foods and to determine whether residual radioactivity is a problem.

In initiating any new process for foods it is always necessary to determine whether nutrients are destroyed and whether toxic substances are formed. Information available at the present time indicated in general only a small loss in nutritive value for the foods investigated and gives no indication of the formation of toxins. Taking into account the nature of the ionizing radiations used in the irradiation process, however, it is reasonable to be more than ordinarily militant about possible changes in these two areas. As a result, effective precaution will be exercised in work on this portion of the problem.

9. Research on vitamins and other food components to determine the extent of losses due to irradiation.

Research on vitamins will, of course, be very similar in scope to the other categories in this group which deal with the mechanism of chemical changes due to irradiation. Work on other food components in this area may be centered on the use of purified chemicals such as proteins, amino acids, etc.

10. Determination of the quality and acceptability of irradiated foods.

Determination of acceptability is a dominant note in most of the research carried on at the Quartermaster Food & Container Institute. It is equally important in this project because ultimately the utility of any process involving irradiation will be measured directly in terms of acceptability of the final product to the military personnel using it.

11. A study of the chemistry, microbiology, and acceptability of irradiated foods in long-term storage.

This is another familiar item in Army ration research. Its emphasis arises from necessities for long supply lines, far-flung storage operations, and widely varying climatic conditions encountered in military operations. All these things combine to emphasize the need for food items with high stability for military use. It is very likely that the same criteria that usually are used in judging new developments for the stability of Armed

Forces rations will be applied in this case.

12. Evaluation of present containers and study of new containers suitable for radiation processing.

It is difficult to guess at the moment what the requirements will be for containers for irradiated food products. In common with other processes however, a container will be necessary which will prevent recontamination of the food after processing and which will allow most efficient use of the radiation process in promoting sterility. For this purpose it may be necessary to investigate the use of plastic or other special containers which may be found to have especially favorable characteristics.

13. Investigation of asceptic processes for use in conjunction with irradiation.

One possibility which presents itself in connection with irradiation is the use of asceptic filling and closing operations to be applied to the already processed food item. At the present time and with conventional processes this method has found use primarily with liquid products but might conceivably be used for solid or semi-solid items which have been rendered sterile by irradiation.

14. Provision of radiation service to collaborators in the project.

This is not a research category but has been set up instead to service research teams and provide them with irradiated material which has been treated according to the needs of the experimentor. Difficulties in carrying out such a supply operation are many and are quite plainly apparent. The alternative, however - that of having adequate radiation facilities at each location where research is done - is at present certainly no more attractive. A great amount of effort will have to be placed in this area to assure a smooth and effective operation.

From these fourteen areas four were selected for immediate activation. These are:

1. Mechanism of off-odor and flavor development.
2. Toxicology.
3. Technological studies.
4. Supply of radiation energy.

Mechanism of off-odor and flavor development has been sub-divided where possible, to give emphasis to research on the important food components such as fats, carbohydrates, and proteins. Each part of the work is being done by research teams possessing special capabilities in one of these fields.

The objective is to find out which chemical systems contribute to quality losses and the mechanism of the changes involved with a view to finding methods for their prevention.

Studies of toxicology and nutritive changes are being conducted in direct cooperation with the Office of the Surgeon General, U.S. Army, since this group has the responsibility for the protection of the soldier's health and in addition has the trained personnel necessary to carry on these studies.

Technological studies are designed to include work on the effects of the physical characteristics of the source and the method of treatment on food commodities as well as investigations of stability and acceptability of the final product.

Energy level, dose rate, geometry of the source, effective intensity, and the duration of irradiation treatment are under study. Results avail-

able at present have been accomplished using only relatively low intensity sources over long periods of treatment. With the higher intensity sources now available, it is hoped that more rapid progress may be made and, in addition, that the effects of the physical characteristics of the source and method of treatment may be catalogued for all of the food items of importance in Army rations.

Evaluations of irradiation processes require for their implementation sources of supply of food materials treated according to experimental plans evolved by collaborators in the program and designed to fit a specific pattern of research. It is intended that this supply operation will be maintained by the Quartermaster Food & Container Institute in close cooperation with individual research groups.

Within the next fiscal year, efforts will be made to activate virtually all of the areas of interest originally outlined, as well as to continue support of those areas now in action.

As soon as research makes processes available for preparation of a highly acceptable food product, these processes will be tested on a commercial scale to determine industrial feasibility.

The economic picture, while not yet complete, suggests decreased costs for both machine and fission product energy.

If decreased energy costs can be coupled with savings in quality resulting from radiation sterilization of food commodities, the result should be an important advance in food preservation.

IV

# MEAT IN SPECIAL DIETS

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My assignment in this conference is to discuss meat in special diets, with particular reference to the question of the possible need for commercial products which are designed to help physicians and dietitians in the treatment of their patients by dietary means. There is of course no simple answer to this question. It is a matter which demands continuous study and research, but a consideration of the present role of meat in normal as well as in therapeutic diets may help direct the thinking of those of us in the food industry into useful channels. By "meat" is meant "red meat" in the usual sense of the term. But in the larger sense, nutritionists consider that meat is one of a group of important foods of animal origin, which includes milk and its products, fish, poultry and eggs. Consequently, while our attention is now confined to meat and meat products, the views expressed pertain in some measure to all products of the great group of animal protein foods.

## Dietary Significance of Meat

That meat and meat products are important foods in the American dietary is well understood. Although the per capita consumption of meat in the United States is considerably less than that of certain other meat producing countries such as the Argentine, New Zealand and Australia, and somewhat less than the per capita consumption in Canada and in several European countries under normal conditions, people in this country commonly think about meals in terms of meat. It is so with the menus in our restaurants, and it is so in our homes, where only after the meat is chosen - by the homemaker to please her husband's taste and pocketbook - are the accompanying vegetables and other foods selected to make a meal. Because of truly gigantic and well-functioning systems of production, storage, and distribution, meats are available to consumers in ample quantities and in many different forms. Meats can be prepared in numerous attractive and appetizing ways. The savory aroma of cooked meat stimulates the appetite; and no other food excels meat in the production of the physiological sense of satisfaction, or satiety, which follows its eating.

As Kraybill (1948), Aitken (1950) and others have pointed out, the composition of meat shows that it is of high nutritive quality. Meat is an outstanding source of protein, and it is an important contributor to the diet of riboflavin, niacin, thiamine and other members of the vitamin B complex, and of iron. Although the consumption of ample quantities of meat does not of itself quarantee dietary adequacy, it is significant that malnutrition when encountered in the United States is usually associated with a marked reduction in the consumption of this food. It would be difficult to devise a nutritionally adequate and satisfying diet for prolonged consumption by adults without the use of meat; certainly this assignment would not be undertaken without considerable misgivings by most of our nutritionists.

Meat is highly digestible. Properly selected and prepared as they are,

canned, sieved meats are well tolerated and digested by infants, even by those prematurely born. The popularity of canned, sieved and chopped meats in the feeding of infants and children reflects favorably on the entire meat industry. I am inclined to think that this popularity has a psychological basis rather than a nutritional one. Parents are interested in the growth and development of their babies; it must give a sense of satisfaction to mothers to be able to say that the baby at an early age is eating meat, specially prepared for his needs, of course. Physicians are satisfied that the sieved meats provide good nutritive values, and the investigations which have been sponsored by industry have provided the evidence on which such an opinion can be based. The advantage of sieved foods, from the pediatrician's point of view, is in the help they offer in the transition of the infant from nursing in his mother's arms to the taking of his place at the table, as a full fledged member of the family. Proper emphasis on the nutritive values of meat in the diet of the infant can thus lead consumers to a fuller appreciation of the nutritive value of meat in the diet of the adult.

The characteristics of meat which make it so important as a food in normal diets also commend it for use in special diets. The expression "Special Diet" means a diet of relatively known composition which is designed for a particular purpose. It may be a diet to meet the needs of a particular age group, such as infancy or old age. It may be a therapeutic diet for use in the dietary management of disease or abnormal condition. Commercial foods, the ingredients of which are so selected, formulated or processed as to make them useful in special diets, may be called variously "Special Purpose Foods," "Foods for Special Dietary Use," or "Dietetic Foods." It is expected that technological skill and scientific control over the production of dietetic foods will be exercised. It is required that the labels of these foods inform purchasers of the special features of each product, such as its typical composition as shown by analysis, and indicate its recommended or suggested uses in various special or therapeutic diets.

## Special Diets

The concept of special diets has undergone considerable change in the last 15 years, coincident with our greatly increased knowledge of nutrition during the same period. These changing views have been duly reported by Boller, Hunzicker, and others in articles written for "Food and Nutrition News," the valuable publication of the National Live Stock and Meat Board. Dunlop, a physician of Edinburgh, has described (1950) the therapeutic diets of some 15 years ago in the following manner:

> "We had diets for hypertension, Carrel diets for heart disease, diets for rheumatism, diets for every stage of Bright's disease, ketogenic diets for urinary infection, step-ladder diets of alarming complexity for diabetes, Gerson diets for pulmonary tuberculosis, elimination diets for asthma and allergic states, and, of course, a bewildering number of diets for various types of dyspepsia to which we usually gave names: the Lenhartz diet; the Sippy diet and Hurst's modification of it; the Meulengracht diet and Witt's modification of it; Schmidt's intestinal diet; the pulped-apple diet; Sprigg's lacto-vegetarian diet, and many others. We were apt to look upon these diets as entities in themselves, prescribing them rigidly to the bank clerk, the night watchman, the stockbroker and the miner. Simple scientific principles were often lost sight of, and fashion and tradition made numerous impositions, but particularly restrictions which were very often unjustifiable on any rational basis. Our diets were almost always inconvenient to the patients and often actually detrimental to him from the point of view of sound nutrition."

Truly, fancies and foibles regarding foods and nutrition are not limited to the general public or to the promotors of so-called health foods; this assertion is made without necessarily criticizing the several diet plans mentioned by the Edinburgh physician, for some of them are still in good repute, especially when modified to accord with modern views of nutritional requirements, and each of them doubtless served a useful purpose at some time. It is of interest that Dunlop did not mention the early liver diets of Minot and Murphy, which marked the first use of a meat product for therapeutic purposes. Liver diets were effective in the treatment of pernicious anemia, but 15 years ago we had liver extracts for parenteral use, and today we have preparations also of vitamin $B_{12}$, which is the agent in liver that is principally responsible for the effectiveness of this meat product in the treatment of a disease which, before the time of Minot and Murphy, was incurable. Newer chemotherapy, and the development of antibiotics, have made some older therapeutic diets no longer necessary.

Today, we try to think of the principles of diet therapy, and we try to make our diet instructions simple, flexible, and altogether more practiable than they were in previous years. The objective in the dietary treatment of the patient, is to prepare him better for surgery, or other treatment, and to speed up convalescence, if possible, so as to discharge the patient sooner from the hospital, and in better nutritive condition, and to provide him with suitable dietary instructions, when it is desired to continue the dietary treatment at home.

For our purpose of considering how, as representatives of an important food industry, we can help in this overall objective, it will be worthwhile to think of the relative importance of various nutrients in the treatment of the hospitalized patient. For the convalescent patient, who has undergone a surgical operation or, who is responding to medical treatment of infectious or wasting diseases, the immediate need is for water and certain inorganic salts which are present in body fluids. The next important requirement is for calories. It may be necessary to administer fluids containing electrolytes and simple sugars, such as dextrose or levulose, by vein. Amino acids, usually in the form of hydrolyzed proteins, are often administered, because of the desire to prevent further depletion of the body's supply of proteins and because of the demonstrated value of the administration of protein hydrolysates in many conditions. Some progress has been made in the administration of fats by the parenteral route. All these measures, however, are of emergency or temporary value. The desire is to bring the patient back to the normal living condition, where he has an appetite for meals, and where he may select his foods according to his particular needs.

The basic principle of all therapeutic diets is that they shall meet the nutritional needs of the patient while, at the same time, they shall have the other features which are desirable for the dietetic therapy of the condition being treated.

Sometimes it is necessary to avoid certain foods, in the treatment of gout, where glandular and organ meats are proscribed because of their relatively high content of purine substances, which are precursors of uric acid. Similarly, salted meats and other salted foods are prohibited in low-sodium diets. In general, however, the emphasis is on the important "do's" for the selection of an adequate diet, with relatively few "don't's."

While the principles of therapeutic diets are relatively simple, putting them into practice oftentimes requires considerable patience and ingenuity. It is in this realm that all persons who are engaged in the processing of foods, their preparation for the table, or the actual planning of meals, are called upon to exercise their respective skills and ingenuity to make the meals attractive and at the same time have them meet the medical requirements for the diet as a whole.

## High Protein Diets

An example of the nature of the problem is provided by the experience of Kountz, Hofstatter and Ackerman, who observed negative nitrogen balances in a group of elderly persons. When they improved the diet, by the addition of substantial quantities of eggs, skimmed milk, cottage cheese and beef, they found that the patients refused part of the food, thus defeating the purpose of the dietary alterations. Recourse was had to the inclusion of a hydrolyzed protein preparation, derived from dried yeast, which could be incorporated in different items of the diet without detection by the patients, and for that reason it was successful.

To develop a diet unusually rich in protein of high nutritive quality, which patients will consume regularly, is not a simple matter. This problem has been studied comprehensively by Spinella and her colleagues of the Army Medical Nutrition Laboratory in Chicago. Their first report, in 1947, described their experience in providing up to 200 grams of protein in therapeutic diets of patients with insufficient appetites for ordinary foods. They attempted to incorporate extra protein in as many regular foods as possible, without altering the bulk of the food. In other words, they tried to improve the diet, while at the same time the customary foods remained normal in appearance and taste; acceptability of the product by the patients was the principle aim, although, of course, the cost factors had to be borne in mind. They found that the protein content of the diet could be increased from 30 to 100 per cent without affecting flavor, taste, appearance or bulk, through the use of natural proteins, concentrates or hydrolysates, as ingredients of various recipes.

Later reports have shown that the most useful protein-rich ingredients for use in the preparation of high-protein dishes are calcium caseinate and dry skimmed milk. To a lesser extent, canned, sieved meats have been found to be useful in the preparation of certain foods. Spinella has summarized the experience of several years in a publication (1952) entitled "Recipes and Menus for High Protein Feeding." This publication provides 74 recipes for large scale preparation of many dishes, the protein content of which is increased through the use of calcium caseinate, dry skimmed milk, or canned, sieved veal; a number of additional recipes are provided for the preparation of foods in which the protein content is increased by judicious alteration of the normal ingredients.

The reports of the advantages of high protein feeding raise the question of how much protein is considered to be a desirable high level of this nutrient. Spinella, Imperiale, Hofer and Sargent (1950) studied the value of high protein diets in the management of rheumatic fever. Their patients were 15 children who were studied over a period of 28 weeks. The ordinary diet of these children was found to be nutritionally adequate with respect to its protein content, both qualitatively and quantitatively. It is not surprising, therefore, that the administration of a "high-protein" diet to these children did not have any observable influence on the rheumative process during the period of study.

It is likely that high-protein diets will be most useful when there is a history of previous protein insufficiency, or when the demand for protein is unusually great, as it probably is during convalescence, in patients with severe burns, and for blood production and wound healing. Much can be accomplished with ordinary foods, or with a relatively few protein foods, but additional work could still be done on the problem of devising a palatable high-protein diet, which provides 2 grams or more of protein for each kilogram of body weight. The concentrates which have been employed most successfully, namely, calcium caseinate, dried skimmed milk and canned, sieved veal, are limited in such uses; their value is mainly restricted to high-calorie diets.

The requirements for various nutrients during illness and convalescence

have not been established. Regarding protein need, Pollack and Halpern in the N.R.C. publication, "Therapeutic Nutrition" (1952), have written as follows:

> "There is evidence to show that sever depletion of body protein leads to a prolonged convalescence, poor wound healing, an increase in complications after surgery, increased susceptibility to infection and anemia, hypoproteinemia, edema, reduction in enzyme activity, disturbed gastrointestinal motility, muscular weakness, fatigability and asthenia, and a markedly reduced tolerance for blood loss. In experimental animals, liver injury can be demonstrated after protein depletion. A vicious cycle may be started if the protein deficiency leads to anorexia."

Yet there is little evidence available about how much protein is required in the treatment of any of the conditions mentioned. The tendency is to give as much protein as possible.

## Other Therapeutic Diets

Because the emphasis is placed on having all diets supply at least the recommended allowance for various nutrients for persons in good health, frest meat is usually included in therapeutic diets. Most low-calorie diets include meat; meat is allowed for in the dietary instructions given to diabetics; and fresh meats are included in all except the most severely restricted low-sodium diets. The principle restrictions to the use of meat are those which have been mentioned, namely, the avoidance of glandular and organ meats in the treatment of gout, and the avoidance of salted meats in planning low-sodium diets. One field which is being actively studied is that of the role of cholesterol, and of the fat level of the diet, in atherosclerosis. Much remains to be learned, but at the present time, the prevailing opinion is that foods which normally have a high content of cholesterol, such as meat, milk and eggs, are so valuable as sources of important nutrients, and the evidence regarding the effect of cholesterol is so controversial or incomplete, that it is wise not to restrict the use of these foods because of their cholesterol content.

## Low-Calorie Diets

The use in reducing diets of liberal amounts of protein, supplied in large measure by meat, was described by Campbell as long ago as 1935. The diets which he used provided about 2 grams of protein for each kilogram of body weight. The actual diets described in his report provided from 1,335 to 1,758 calories daily, and from 101 to 132 grams of protein. On these diets 27 patients lost from 1.2 to 6 pounds each week for periods of from 4 to 50 weeks.

Normal quantities of lean meat and related foods are now customarily recommended, as has been stated, in various other dietary plans for reducing weight. There is a need, however, for simplified instructions in low-calorie diets. Particularly there is a need for suggested meal plans that take cognizance of the desirability of meat-free days for some persons who are asked to adhere to a low-calorie diet during Lent. Fish, poultry, and eggs can be used, of course, and this fact should be recognized. The inclusion of a few meat casserole dishes, including macaroni products, would make many of the dietary suggestions for reducing weight more practical. These are assignments for the dietitian. Interested segments of the food industry could well take a broad view of promotional materials which they develop about the use of their products in low-calorie and other therapeutic diets,

with the view of making such material as usefully informative as possible.

Low-Sodium Diets

The value of low-sodium diets in the management of hypertension and of congestive heart failure and other conditions where there may be a tendency to the formation of edema fluid, has been generally accepted. Not all patients are relieved by such dietary restrictions, but in those who are, the benefits in the form of increased sense of well-being are tremendous. The so-called rice diet has been used with clinical success for short periods of time. However, the American Heart Association, in its manual for physicians and patients (1952), recommends a diet patterned after the ordinary American dietary, and stresses the importance of nutritional adequacy of the diet and the importance also of weight reduction when necessary, to attain the desirable body weight for the patient's age, sex and degree of physical activity. Unsalted meats may be used in mildly restricted sodium diets (sodium content 1.5 to 3 grams daily) but not salted meats, such as ham, bacon, dried beef, bologna or sausage. Fresh meats are included in the moderately restricted diets (0.5 to 1.5 grams sodium). In severely restricted diets (less than 0.5 gram sodium) animal protein foods must be rigidly controlled, because of the relatively high natural sodium content of these foods. Milk, eggs and meats (including fish and poultry) contain about 50 mg. to 100 mg. of sodium in each 100 grams. Ordinary cheese is high in sodium content also.

Yet even in the severely restricted low-sodium diet, recommendations call for 3 or 4 ounces of fresh meat daily, and one egg or an additional ounce of meat. For children, or for adults, commercial milk preparations which have had the sodium removed by a base exchange method, or be dialysis, are suggested. The severely restricted dietary regimen may require supplementation with vitamin and mineral preparations.

There are a few commercial meat and fish products, made low in sodium content, which have been introduced. The Council of Foods and Nutrition has considered several of these products, and has called attention to the desirability of making known the loss of other nutrients, such as niacin, riboflavin, and thiamine, in those foods which have been processed so as to be low in sodium, and in the processing have suffered unusual losses of these nutrients.

Ferree (1953), in providing the conclusions of a special committee of the American Heart Association on the labeling of low-sodium foods, has mentioned the desirability of declaring the sodium content of these products. It is expected, as a result of recent hearings of the Food and Drug Administration, that this recommendation will be made a requirement. The question of how much sodium might be permitted in a food product, in order to warrant its being called a low-sodium food, was also considered by this committee. For meats, fish and fowl, this value is 100 mg. of sodium per 100 grams. For fruits it is 15 mg., and for vegetables, 20 mg., per 100 grams.

The National Canners Association and the Can Manufacturers Institute, however, in their study of low-sodium canned fruits and vegetables (1953) have shown that most commercial canned products are considerably lower in sodium content than the maximum figures permitted by the committee. They have pointed out to the canning industry various sources of possible contamination with sodium in processing. This industry-sponsored study is proving of considerable practical value. This program should be of interest to the meat industry, because meat can be used with greater assurance in low-sodium diets when the other components of the diet are as low in sodium as it is possible to produce them.

A recent article by Kisch (1953) could well be studied because it points put one of the practical difficulties which physicians may experience when they make suggestions about therapeutic diets to their patients. This author

points out that the prescribing of a low-sodium diet may cause difficulties for persons who adhere strictly to Jewish dietary laws. These laws demand the salting of fresh meat before use. Kisch described some experiments which show that the sodium content of meat so treated may be returned to the normal value by cooking the meat in a generous amount of water and discarding the broth.

There is doubtless opportunity for additional study to determine if certain meat products can be commercially prepared so as to be acceptable to the taste, low in socium content, and provide the expected nutritional values of meat. Luncheon meats low in sodium would be a boon, in the opinion of some of the dietitians. Perhaps by the judicious selection of seasoning ingredients acceptable products might be produced.

V

# RECENT DEVELOPMENTS IN NUTRITION RESEARCH

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It is a pleasure to meet with this group and to note the excellent progress that is being made here, under the direction of Dr. Henry Kraybill and his associates at the American Meat Institute Foundation. There is no doubt that an audience such as this has an interest in a lusty set of twins growing up in the industry—namely, "good food," paired with "good nutrition."

## Absurdity of Present Conditions

As a nation, we are in a most illogical position. We are envied by the rest of the world for the abundance, convenience, variety, attractiveness, sanitation and year-round availability of food that is well within the economic reach of almost our entire population. We are even embarrassed by the grossly wasteful practices that result from a so-called "surplus" of foods, and still more embarrassed by the conclusion of competent scientists, that somewhere in the range of 20 to 25 million individuals among our population severely handicap their prospect of maintaining good health, by eating more than their bodies need (in terms of calories) with resultant overweight and damage to their hearts, blood vessels, livers, and other organs.

Despite recognition of the general nature of the problem, pressure groups working behind the scenes in legislative committees and in other parts of our political-economic structure prevent us from taking steps to develop efficiently our food resources.

Another relatively absurd situation prevails in that a large fraction of our population, when studied as individuals, does not consume diets that are satisfactory in quality, as expressed by their content of amino acids, vitamins, mineral elements and fats. They do not obtain enough of all the desired nutrients in the sense of optimum health among mothers, small children and especially the older age segments of our population.

There is good reason to believe that imbalances and partial deficiencies of essential nutrients magnify the degree of injury that is associated with an intake of excessive calories. As a matter of fact, we do not have an adequate picture of the exact nature of our dietary faults. New information is developing steadily, but there is little room for doubt that bad practices currently contribute to the increasing incidence of the so-called degenerative diseases, especially among the older people within our population. Fortunately, we have escaped almost completely from the period when clear-cut, severe deficiency diseases were common, as illustrated by scurvy, rickets, pellagra and goiter. These conditions only occur, however, after the body has been subjected to damaging deficiencies through a long period. Although the principle is well known, that lesser deficiencies are definitely injurious, we have very incomplete measures of their current effect upon our human population. The combined evidence from human experience and controlled tests with animals, however, justifies one's confidence that dietary faults still constitute a severe strain on our health records.

## Research Gives Answers

Answers to the problems will come primarily from intensive research to gain a better understanding of the composition of foods and the functional role that all of the nutrients play in living organisms. This kind of basic approach includes the use of a great variety of living organisms, ranging from the simplest plants and animals to man. A second type of research is geared to practice, and serves to guide both the food consumption practices of our population and the food production policies in agriculture and manufacturing. These essentially practical aspects of food use are far more important than generally recognized in food merchandising, as in stores and restaurants.

Our leading authorities in professional groups that are alert to the nation's public health problems at the present time, including university personnel, the officers of scientific societies, the U.S. Public Health Service, the medical societies and the statisticians of insurance firms are all in agreement that we are passing rapidly from the age-long period (essentially all of man's experience until the last few decades) when infectious diseases constituted the greatest threat to health. Many of the virus diseases are still uncontrolled but even in these cases the research outlook is improving rapidly. Poliomyelitis serves as a striking example where rapid progress in research is underway.

We are already in a period when four of the five highest causes of death among American citizens (and citizens of other countries in comparable stages of technical advance) are associated chiefly with imbalances or derangements in body metabolism. At the top of the list is heart disease, next comes cancer, then the diseases associated with injury to the blood vessels as witnessed in cerebral hemorrhages or strokes, diseases of the new born can be grouped in fourth place, and finally we reach a group of diseases caused by infections, in which the pneumonias and influenzas are grouped together. Diabetes, arthritis, liver disease, kidney failures, ulcers and many other diseases of the chronic, metabolic type are also high on the list that is costing us heavily in human resources. Reliable public health leaders predict that unless the present trend can be changed, we are likely to have a 50 per cent increase in the incidence of the chronic, disabling diseases within the next 20 years. They report, for example, that among the trends during the past half-century, we have increased the life expectancy at birth by more than 18 years, but for those at age 50, the life expectancy has been improved by only about two years. This same pattern of relative improvements in health among children, in contrast to lesser gains in the upper age brackets, is found in the countries of western Europe.

The urgency of getting more information about the chronic diseases associated with maturity and old age can be pictured in another way by pointing out that although our total population has approximately doubled during the past 50 years, our population above age 60 has quadrupled. Another area of increasing importance among the metabolic diseases pertains to the incidence of unbalanced mental states. In this area of correlating mental diseases with physiological disturbances, very little progress is being made.

## Specific Advances in Protecting the Health of Infants and Children

Blindness or near blindness among prematurely born infants in recent years has become a serious problem. It has been associated in a peculiar way with the premature infants born within a fairly characteristic weight or age group. The condition (retrolental fibroplasia), as a health problem, appeared to be of recent origin, and it also showed a wide variation from community to community and from year to year. The condition could not be

associated with any evidence of infection, so pediatricians continued to search for metabolic or nutritional factors that might be at play—possibly conditions imposed by failing to know how to feed premature infants. One theory after another was proposed as a possible cause of the tragic condition. Recent evidence seems to point convincingly to the conclusion that the disease is not caused by malnutrition, but instead, by supply to the premature infant, at a critical stage in their development, an excessively high concentration of oxygen in the incubators. It is a common and beneficial practice, of course, to protect premature infants by an extra supply of oxygen, but it was a surprise to find that the eye tissues could be so sensitive to irreversible injury by an excess of oxygen, alone.

Dr. Gyorgy at the University of Pennsylvania has made a notable contribution in studies of the beneficial effect of colostrum. A newly identified factor is present normally in high concentration in human milk but it is especially high in colostrum. It is present also, but generally in lower concentration in the milk and colostrum of other animals, where it induces a favorable flora in the intestinal tract and hence appears to give the infant a distinct physiological advantage. The structure of the compound has not been reported.

Dr. Gyorgy and others have made substantial contributions also in the studies of nutritional factors that can be beneficial in warding off fatty and diseased livers. This represents an area of considerable significance. Correlations between fatty livers early in life and later breaks in health have wide interest, and the disease kwashiorkor (in which fatty livers are a conspicuous feature) is one of the most prevalent diseases among infants and small children in tropical areas where the food intake is excessively high in carbohydrates and very low in good quality protein. The degree to which injury by low quality protein intake is further aggravated by a low intake of vitamins and minerals has not been adequately explored. Nearly all authorities on nutrition in tropical and subtropical areas agree that this kind of problem is one of the most severe and widespread with which they have to contend.

Notable advances have been made also in identifying the importance (and sharpness) of the time factor in causing congenital malformation when the mother is subject to one or more deficiencies. The classic work of Dr. Josef Warkany and his associates has been extended by many other investigators with comparable results. In a review of advances in this area of nutrition, A. G. Hogan (Annual Review of Biochemistry, 1953) pointed out that reliable reports are now at hand showing that a great variety of deficiencies, including vitamins, minerals, amino acids or a simple deficiency of oxygen, during the respective critical stages of gestation can result in a high incidence of congenital malformations. These injuries are generally most evident in the skeletal structures, the central nervous system, glandular tissues, the heart and the eyes. Unfortunately we do not have enough evidence to give a reliable picture of the relationships of this kind among our human population. The reports by Dr. Harold Stuart, Mrs. Burke and many others give us at least good reason to think that in this area of human nutrition, distinct advances in human health probably could be made. The data from medical records in such typical areas as Boston, Philadelphia, Aberdeen, Edinburgh, New York and Chicago indicate, however, that the incidence of congenital malformations is in the range of 1 to 3% of live births. The statistics, however, are not well standardized for clear interpretation.

Another significant advance has come from the discovery that crude table salt, as produced directly from sea water in tropical areas, was very likely to be deficient in iodine. The iodine compounds initially present in the sea water are usually lost during slow evaporation to obtain crude salt. Furthermore, if the formula used for the iodization of pure salt (such as we

are accustomed to) is used with crude salt, the iodine is quickly lost. Hence, it was an important forward step this year to find that iodate could be added to the crude salts, with satisfactory stability in the product and with equally effective prevention of goiter (Dr. Nevin Scrimshaw, et al). These findings were reported from Guatemala. Here you have another good example of modern technology furnishing a product that has health advantages over the crude product used by primitive peoples.

We have had very little information about the significance of vitamin $B_6$ in human nutrition. There have been occassional reports based on only a few patients. This relatively new vitamin came sharply into the limelight this year, however, when it was found that some of the formulas in common use for infant feeding had been over-processed or modified in composition to a degree that acute deficiencies resulted. Cessations in growth were accompanied by occassional convulsive seizures. Fortunately the risk on this score was quickly identified and has been corrected, I believe, by all of the infant-food manufacturers.

## Tooth Decay

In the case of fluoride intake, it is worth remembering that only a few years ago such a renowned investigator as Professor McCollum at the Johns Hopkins University sought to find whether they could establish an essential nutrient role for fluoride as a trace element. The findings were negative. Now we have the contrasting situation in which small amounts of fluoride supplied by automatic controls in community water supplies (at a concentration of 1 p.p.m.) furnishes approximately a 50% reduction in the incidence of tooth decay or dental caries. Of course the advantage must start with small children in communities where the fluoride content of the water has been less than 1.0 p.p.m., but this range of concentration includes a large fraction of supplies for our total population. The advantages derived from fluoridation of water supplies have been established with sufficient clarity, and with no apparent risks of injury, that approximately 1,000 communities in the United States have now adopted fluoridation and have installations either operating or under way. Any reasonable evaluation of the evidence regarding the great gains in health, balanced against an extremely small cost and the absence of any apparent risks to health, can scarcely fail to justify approval of fluoridation as a forward step of first rate importance. Few people realize that the costs for dental care now amount to about 20% of the total non-government cost for personal medical care in the United States. So we are dealing with a problem of major magnitude.

Somewhere in the picture, there is still a serious need for finding a safe and inexpensive way to provide an adequate fluoride intake to those who do not have access to controlled public water supplies. The fluoride content of bones and related products should furnish a clue.

Dr. M. O. Schulze at the University of Minnesota has reported interesting observations on one or more nutrient factors that exert a favorable effect on lactation in experimental animals. These reports remind us of earlier findings from Japanese laboratories, where two lactation factors were described in the form of crude concentrates a number of years ago, but their identity was never established. Continued efforts to isolate nutrient material of this kind will be watched with great interest. There is good reason to believe that such products as liver, yeast and milk contain more than one constituent to be identified still as vitamins.

## Research Advances Pertaining to Upper Age Brackets

Dr. C. A. Elvehjem and his associates at the University of Wisconsin have reported this year a number of interesting relationships in which the

amino acid balances are of critical importance in protecting the livers of experimental animals against excessive deposits of fat. This whole area of discovering the injuries induced by acute and chronic imbalances represents one of the most important aspects of current nutrition research.

The group associated with Dr. Elvehjem has likewise reported important findings that point toward the urgency of maintaining balances in the mineral intakes, e.g., of calcium, magnesium and potassium. Although there is substantial evidence that additional vitamins and trace mineral elements remain to be identified, the research frontier is now characterized more by explorations and discoveries of the importance of interplay or balanced intake among different nutrients rather than the search for nutrients that are strictly essential.

Dr. F. J. Stare's group at Harvard University has made a notable contribution in demonstrating that a deficiency of the amino acid methionine can play an important role in the onset of atherosclerosis in experimental monkeys. The injury induced by the amino acid deficiency is very similar to that observed commonly in the human population, in severe atherosclerosis—injury of the arteries near the heart.

Perhaps no other area of metabolic or nutrient research has advanced so rapidly through recent years as the study of chemical changes in relation to fat synthesis and fat degradation. Primary emphasis is now placed upon the acetate group as a result of the use of radio carbon to trace through the formation of fatty acids, cholesterol and related fatty compounds (from acetate groups). The acetate groups in turn can be derived, also from either fatty materials or from the pyruvate group formed from sugars. The work of Lehninger, Lardy, Bloch, Green, Linnen and others has given a remarkable new insight into this area of nutrition. And the discovery of Lipmann and his associates, that the vitamin, pantothenic acid, constitutes an essential part of the catalyst responsible for many of the changes essential to fat metabolism, has given another major key in nutrition research. Identification of aminomercaptoethane as a fragment conjugated with pantothenic acid (Snell, et al) has extended the chemical concepts of protein and fat metabolism still further.

The fact that excessive deposits of fat in the human body are so closely correlated with accelerated aging, adds very greatly to the confidence one has that these basic discoveries of a biochemical nature will prove to be of great advantage in the improvement of human health.

The channel of reactions leading from glucose to glucuronic acid and ascorbic acid has been explored further. These is extensive evidence now that glucuronic acid (a constituent of animal tendons and other mucoid tissue) is an intermediate between glucose and vitamin C in plants and in animals that are not subject to scurvy. Dr. Karl Meyer at Columbia University has developed evidence also that ascorbic acid and glucuronic acid have a close relationship to bacterial products such as hyaluronic acid. His work has come from an entirely independent line of study, in attempting to follow glucose into the products that accumulate after an infection. An interesting item in relation to the use of vitamin C in recent technology has been the protection that the vitamin can give toward maintaining a favorable color in meat products. The vitamin has been used to protect delicate colors and flavors while processing fruits and vegetables, but its use in meats and fish for this purpose is relatively new.

A classical example of basic research setting the stage for practical applications of great significance in medicine is again on the horizon. Results have not advanced far enough to be certain of their utilization, but it is interesting to note that two of the most promising agents now being tested for partial control of cancer development, are derived respectively from studies of the vitamin, folic acid (antagonist to leukemia), and a purine compound derived

from research on nucleic acids—a mercaptopurine, as reported by the group working at the Sloan-Kettering Institute.

## The Trace Element Molybdenum

Dr. George Davis' work in animal feeding at the University of Florida has included studies of molybdenum, copper and iron, and the interplay of these elements in animals and plants. The work has now been extended to include cobalt as a fourth element of importance in the mineral balance. Meanwhile, parallel studies to identify the nutritional requirements to form an enzyme in the liver (xanthine oxidase) particularly in the work reported from Syracuse University, University of Wisconsin and Lederle Laboratories has identified molybdenum as an essential part of the liver catalyst. Increased attention has been given in recent years, also, to the importance of molybdenum as an essential plant nutrient in the management of vegetable crops. The Johns Hopkins group has observed the element's key role in reducing nitrate nitrogen to lower valence forms, to start the building of amino acids and related products.

One of the most spectacular research advances during the year has been the complete structural analysis of two protein hormones derived from the pituitary gland, followed by synthesis of both hormones from known amino acids for starting materials, as reported by Dr. du Vigneaud at Cornell University.

These are only a few of the landmarks toward understanding how the food we eat contributes to the people we become—or fail to become—according to our life span habits in nutrition practice.

VI

# DIET IN RELATION TO HEART DISEASE

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With a title as general as the one assigned to me it is certainly necessary for one to restrict his remarks. I certainly do not pose as an expert in heart disease or cardiology.

For purposes of general discussion one probably can divide heart disease into three main categories: (1) congenital, in which defects in the anatomy of the heart are responsible and of which a well-known example would be the so-called "blue babies" who have an open connection between the two sides of the heart, which the normal heart does not have; (2) infectious, of which the most common example would be rheumatic fever, where there is an infection, commonly affecting the tissue of the mitral valve and usually associated with a streptococcal infection; and (3) metabolic, a term usually not used in describing heart disease (except that due to hyperthyroidism) but in which may be included atherosclerotic and arteriosclerotic heart disease. A specific example would be coronary heart disease.

It appears to us that the designation "metabolic" for this group of diseases furnishes a more adequate clarification than the usual label of "degenerative." The experts cannot agree among themselves as to the meaning of the term "degenerative." In any event the term is an attempt to describe a disastrous result. The term "metabolic" makes some implications about causation, and this is desirable. It implies that the structural changes found in the heart and great vessels are the consequence of disturbed metabolism either of only those tissues or of the entire organism. It implies that the clinical events are consequences of those lesions, and it suggests that clinical events might be prevented or modified if we knew the fundamental nature of the metabolic defect. We proceed in our research with the hypothesis that a metabolic defect exists and that it is either produced or significantly influenced by the diet.

While it is always attractive to formulate a simple cause of disease, it appears far more likely that atherosclerosis, a metabolic type of heart disease, is the result of several diverse causes, of which diet is one. A number of our researches have led us to recognize the importance of this principle of "multiple causality" in many diseases.

To the extent that overweight adds to the work of the heart for a variable period of years, it is an added hazard in all types of heart disease.

Under certain conditions there is a direct relationship between body weight and the levels of lipoproteins and cholesterol in the blood. This was shown in cooperative studies of our laboratory with physicians at the Peter Bent Brigham Hospital, Boston, and has been observed in the Framingham Heart Study. This suggests that overweight is a greater hazard in metabolic heart disease than simply causing more work for the heart. It may be necessary to distinguish between the hazard of growing fat and that of being fat. We are inclined to think that the former, i.e., the periods of positive calorie balance, of weight gain, are more damaging than is the mere carrying

around of excess fat. This distinguishes so-called active from static obesity. There is so little information on these matters that new and careful research is needed. Obviously this matter is of first importance to the American public.

As many of you know, our Department has been active in a variety of researches on obesity. I do not intend to discuss obesity, but I should like to mention one point about it, and that is that our various studies have convinced us that obesity is simply the end result of a number of different causes involving different mechanisms.

Among other things we have studied the retention of $C^{14}$ from $C^{14}$ acetate incorporated into the diet in the same concentration in pair-fed experiments with hereditarily obese mice, mice made obese by injecting goldthioglucose, and rats obese as a result of hypothalamic injury. Each group was pair-fed with control animals of the same age who were not obese and hence were not gaining weight. The hereditarily obese mice retained much more $C^{14}$ in the fats of the liver and carcass than their controls, thus showing a difference in acetate metabolism. The acetate was synthesized into fat in larger proportions, rather than being catabolized. The mice made obese by goldthioglucose injection did not show any impairment in acetate catabolism, and the rats made obese by hypothalamic injury showed a retention of $C^{14}$ in the carcass fats but not in the liver fats.

These differences in a basic reaction of fat metabolism; i.e., the metabolism of $C^{14}$ acetate, indicate that the positive energy balance in the three types of obesity are due to different mechanisms. Yet the end result is the same in all three types—that is, overweight.

Next I should like to briefly mention a study we have done in collaboration with colleagues at the Peter Bent Brigham Hospital in which we have investigated the influence of weight loss upon the serum cholesterol and upon certain classes of lipoproteins in a group of thirty-nine human subjects.

The subjects were selected primarily according to their willingness to lose weight and return for repeated observations. Twenty-nine of these subjects had cardiovascular disease which served to motivate their cooperation. The remaining ten were well except for obesity.

Several of the subjects with cardiovascular disease reported some degree of cholesterol and fat restriction in their usual diets. In the beginning all subjects were asked to continue the diets to which they were accustomed while taking care to maintain their body weights. Control measurements of serum cholesterol and lipoproteins were made during this period.

After this initial period, caloric restriction was begun on a diet containing approximately 1,000 calories with 100 gm. of protein, 50 gm. of fat and 100 gm. of carbohydrate. The daily ingestion of two eggs was required to insure a minimum daily intake of 600 mg. of cholesterol. By this device a relatively constant cholesterol intake was achieved throughout. The aim was to have the subjects lose at least 2 pounds per week.

Following the period of weight loss there was a period of weight maintenance. During this period the caloric intake was liberalized individually by additions to the carbohydrate and protein components of the diet. Fat and cholesterol intakes were continued unchanged. However, during this period seventeen subjects were instructed to cease the daily consumption of the two eggs and avoid all other cholesterol-rich foods. This regimen was then continued for two months during which time serum lipids were measured in an effort to evaluate the contribution of the egg lipids.

The average weight loss was 19 pounds in each of the thirty-nine subjects, and this led to a significant reduction of the serum lipoproteins and total cholesterol in the majority of instances.

The degree and permanency of this serum lipid reduction can be related to the initial level of $S_f 12\text{-}20$. The influence of weight loss in reducing serum

lipids is greater and more consistent in subjects with initial lipoprotein levels of 50 mg. per cent or more.

The degree of initial obesity is not a determining factor in the serum lipid changes and the rate of weight loss seems only slightly concerned.

Strong positive caloric balance in two subjects on a very low lipid intake was associated with significant increases of the serum cholesterol and lipoproteins. Thus caloric balance appears to play a major role in controlling these serum lipid levels.

On a low fat diet the cholesterol intake had no demonstrable influence upon the serum lipids.

We believe that if elevated serum lipid levels contribute to the causation of atherosclerosis, weight reduction is a proper treatment for this disease.

The last specific study from our laboratory relating diet to heart disease that I should like to mention involves the experimental production of atherosclerosis in a species more closely related to man than the usual laboratory animals. We feel a major accomplishment during the past year has been the development of a technique for the production of atherosclerosis in the Cebus monkey, and I might add it is preceded by an elevation of the serum lipids. This New World primate has been studied in our laboratory for the past five or six years. In the past couple of years we have found it possible to produce in a few months, by dietary means, a form of atherosclerosis which appears to be identical to that seen in humans. The atherosclerosis is produced in the short period of 18 to 20 weeks in adolescent monkeys on a diet low in the sulfur amino acids, high in fat, and with added cholesterol. Neither the lack of sulfur amino acids nor the added cholesterol will by itself produce the disease in the length of time we have studied these monkeys; both conditions must be present.

Our study of atherosclerosis in these primates is the result of an observation three or four years ago when we started out to produce choline deficiency. For this study a diet low in available methionine was devised. In this diet the soybean product known as alpha-protein, which has been shown to have a low level of available methionine, was used as the source of protein. It was observed that inclusion of cholesterol in such a diet led to hypercholesterolemia but only in the choline-fed control animals. Further investigation of this finding has led to a procedure for the production of hypercholesterolemia and atherosclerosis in monkeys.

It has been found that in monkeys in which a high blood cholesterol is maintained by this means for 16-18 weeks, that is, feeding a diet low in the sulfur amino acids and high in fat and cholesterol, lesions of atherosclerosis develop. The condition can be prevented by using a diet with adequate protein or supplementing the diet with methionine, or to some extent, with cystine.

Autopsies have been performed on seventeen animals fed high cholesterol, nine have maintained hypercholesterolemia for 18 or more weeks. Four of these animals showed aortic lesions which were marked, four showed minimal lesions, and one animal was negative both in the gross and microscopically. Of the eight animals on this diet with hypercholesterolemia of less than 19 weeks, there was moderate aortic involvement in one, minimal in two, and five animals showed no vessel disease.

Thus we can say that 8 out of 9 monkeys in which a blood-cholesterol level above 300 mg. per cent was maintained by this dietary regimen for 18 weeks or more developed atherosclerosis. Only 3 out of 8 monkeys developed atherosclerosis when the hypercholesterolemia was maintained for less than 18 weeks. None of the seven control animals showed demonstrable aortic changes.

Summarizing these studies we may say that atherosclerosis has been produced in Cebus monkeys by dietary means. This disease has been produced

by feeding diets high in cholesterol and low in sulfur amino acids over periods of 18 to 30 weeks. Within 2 to 8 weeks this regimen caused the concentration of cholesterol in the serum to rise from 300 to 800 mg. per cent. The hypercholesterolemia could be largely prevented by feeding 1 gm. per day of dl-methionine or l-cystine as supplements to the diet. After the serum concentration had become elevated, it could be restored to normal by feeding dl-methionine but only partially restored by feeding l-cystine daily.

The vascular lesions were in the ascending aorta but extended from the valves to the left ventricle to the proximal portions of the carotid and femoral arteries. Minimal lesions have been observed in the coronary arteries. The lipids were in part cholesterol derivatives.

This study reaffirms the hypothesis that has guided much of our thinking in recent years; namely, that atherosclerosis is fundamentally a metabolic disease, subject to important dietary influences.

In conclusion, I should like to emphasize that the specific researches coming from our Department that I mentioned have all been done by a number of loyal, hard-working colleagues. The obesity research were done by Dr. Mayer and his helpers and the weight reduction studies with patients and the monkey studies by Dr. Mann and his helpers.

When one talks on this subject he is practically always queried on the applications of the findings to man. In this regard one might say that on the basis of present knowledge, the best diet for the person with any type of heart disease is one which provides good nutrition, preferably one that is high in protein and vitamins, moderate to low in fat, and with calories to reach or maintain desirable weight.

VII

# THE NUTRITIVE VALUE OF GELATIN

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The nutritive value or, as it is frequently referred to, the biological value of a protein can be estimated in several ways. Generally the protein to be tested is fed to a group of animals as the sole source of amino acids and the performance of the animals (which may be based on growth, nitrogen retention, repletion of liver protein or activity of liver xanthine oxidase) is compared with the performance of a control group fed a protein of very high nutritive value such as that of whole egg. The results of such trials have made it possible to rate a number of proteins according to their relative nutritive values and to predict fairly well the performance to be expected from animals fed each of the proteins for which information is available. Formulas have also been developed which make it possible to estimate the relative nutritive value of a protein from its amino acid composition. Nutritionally incomplete proteins, i.e., proteins severely deficient or lacking in one or more essential amino acids, show up very poorly when examined by any of these methods.

It should be remembered, however, that the nutritive value of a protein is not an absolute value but depends upon the conditions under which the protein is to be used. Also, estimates based on the use of a protein as the entire source of dietary amino acids may not represent accurately the nutritive value of that protein, especially if it is nutritionally incomplete, as a supplement to a low protein diet. This becomes of particular importance when it is considered that the diets of much of the world's population of both humans and domestic animals are low in protein and that seldom is the intake of amino acids derived from a single food. Gelatin is an example of a protein that is completely lacking in at least one of the essential amino acids and yet can be valuable as a supplement to certain low protein diets.

Since the extent of fat deposition in the liver appears to depend on the adequacy of the dietary protein, we have been studying the deposition of fat in the livers of rats fed low protein diets supplemented with galatin (3-6). The problem arose from earlier studies in our department on niacin-tryptophan deficiency (7,8). When rats were fed low-casein diets deficient in niacin and tryptophan fat accumulated in the liver even though the diet contained what was considered to be adequate choline. The inclusion of either niacin or tryptophan in the diet did not reduce the accumulation of fat, so, in order to study this problem further, the basal diet listed in table I was adopted. This diet is complete in all respects except protein and satisfies the requirements for choline, methionine, folic acid and vitamin $B_{12}$—all of which have been shown to affect fat deposition either directly or indirectly.

Throughout most of the work to be reported weanling rats of the Sprague-Dawley strain were used. The experiments were of from 2 to 12 weeks duration and at the end of the experimental period the animals were killed.

Table I - Composition of Basal Diet

| | % |
|---|---|
| Sucrose | 81.5 |
| Casein | 9.0 |
| Methionine | 0.3 |
| Corn Oil | 5.0 |
| Salts 4 | 4.0 |
| Choline | 0.15 |

Vitamins were included to provide in mg/kg of diet thiamine 5, riboflavin 5, niacin 10, calcium pantothenate 20, pyridoxine 2.5, folic acid 0.2, biotin 0.1, vitamin $B_{12}$ 0.02 and inositol 100.

The fat content of each liver was determined by ether extraction of the dried and ground liver (9).

The results of a preliminary experiment are presented in table II. It should be noted that the fat content of 8 to 10 percent (fresh wt.) in the livers of control animals is definitely lower than that of 15 to 30 percent found in the livers of animals receiving choline-deficient diets. Nevertheless these levels of fat

Table II - Effect of Additional Protein in the Diet on Fat Deposition in the Livers of Rats Fed 9% Casein Diets Containing Choline.

| Diet | Av. rate of gain (5 weeks) | Liver fat | |
|---|---|---|---|
| | | % dry wt. | % wet wt. |
| Weanling rats | --- | 8.8 ± 1.5 | 2.5 ± 0.5 |
| 9% casein-sucrose | 15.5 ± 1.7 | 25.9 ± 2.1 | 8.4 ± 0.8 |
| 9% casein-sucrose + 6% gelatin | 19.3 ± 2.5 | 13.1 ± 1.6 | 3.8 ± 0.5 |
| 11% casein-sucrose | 27.2 ± 1.3 | 14.1 ± 1.2 | 4.1 ± 0.4 |

are considerable higher than the normal values of 3 to 5 percent. Histological studies have confirmed the chemical determinations (10) and evidence of altered cellular structure has also been obtained. Fat deposition was normal in the livers of rats fed the basal diet supplemented with 6 percent of gelatin.

The chart (Fig. 1) shows that there was little fat in the livers of weanling rats. After they had been fed the basal diet for 2 weeks, fat had accumulated to the extent of 25 to 35 percent (dry wt.) and beyond 4 weeks there was a steady decline in fat deposition until at from 7 to 12 weeks the amount of fat in the liver approached a normal value. Although the fat content of the liver decreased as the animals matured, preliminary histological studies have indicated that some focal necrosis remains. It is interesting to note that the decrease in the fat content of the liver parallels the reduction in the amino acid requirements of the maturing animal. However, when rats 10 to 12 weeks of age (weighing between 200 and 300 grams) were placed on a diet in which the protein level was further reduced to 5 percent the level of fat in the liver increased (11). Results of a typical experiment with mature rats are presented in table III. With young rats an increase in the level of dietary protein above 9 percent of casein, and with mature rats an increase above 5 percent, prevented the accumulation of fat in the liver. Thus it is evident that the fat accumulation results from a deficiency of dietary protein.

Figure I - Variation in Liver Fat of Growing Rats Fed Basal Diet.

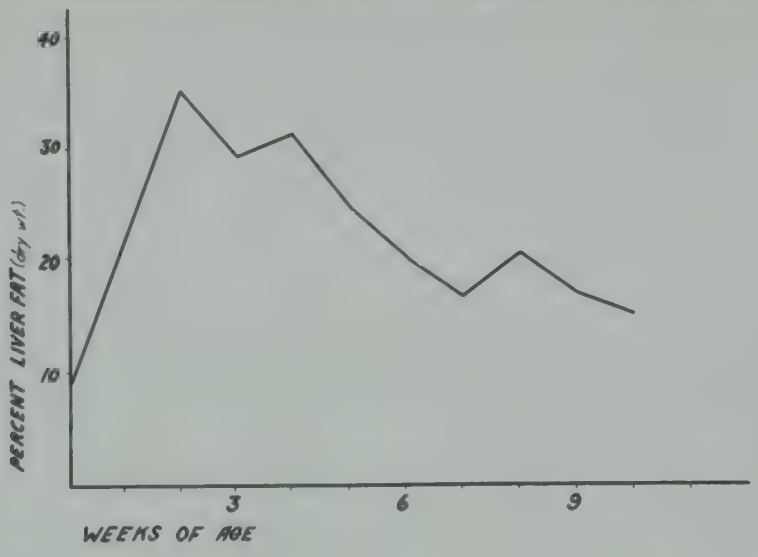


Table III - Deposition of Fat in the Livers of Mature Rats Fed 5% Casein Diets Containing Choline.

| | Change in wt. gm. | Liver Fat % dry wt. |
|---|---|---|
| Basal diet 1 week | -15.6 ± 4.3 | 14.0 ± 1.4 |
| 2 weeks | - 7.2 ± 6.9 | 31.2 ± 5.2 |
| 3 weeks | 0.0 ± 5.3 | 29.7 ± 3.9 |
| 5 weeks | 16.8 ± 6.0 | 31.4 ± 3.3 |
| Basal + 1% choline-chloride 3rd-5th week | 19.2 ± 6.0 | 27.0 ± 2.6 |
| Basal + 0.36% DL-threonine 3rd-5th week | 35.2 ± 1.7 | 17.4 ± 2.0 |

Table IV - Effect of Dietary Choline, Cystine and Glycocyamine on Fat Deposition in the Livers of Rats Fed 9% Casein Diets Containing Choline

| | Rate of Gain (2 weeks) gm./wk. | Liver Fat % dry wt. |
|---|---|---|
| 9% Casein-sucrose (basal) | 15.3 ± 2.1 | 34.6 ± 2.9 |
| Basal + 0.4% choline + 0.5% methionine | 14.2 ± 1.6 | 23.8 ± 1.6 |
| Basal + 1.0% glycocyamine | 12.7 ± 1.7 | 25.2 ± 4.2 |
| Basal. Methionine replaced by cystine | 18.7 ± 1.9 | 28.9 ± 3.0 |
| Basal + 0.18% DL-threonine | 21.7 ± 1.3 | 16.2 ± 1.3 |

That a choline deficiency is not responsible for the fat accumulation is indicated from the results presented in table IV. The inclusion of either cystine or glycocyamine in the diet, both of which aggravate the signs of

choline deficiency, did not increase fat deposition in the livers of animals fed the basal diet which contained choline. In fact glycocyamine caused some reduction in the level of fat in the liver. When the amounts of both choline and methionine in the diet were increased only a small decrease in fat deposition was observed, and in other experiments in which the level of choline was increased to 1.0 percent, 10-fold the stated requirement, the decrease in fat deposition was much less than that observed when 6 percent of gelatin was included in the diet. These observations further indicate that the fat accumulation results from a deficiency of amino acids rather than from a choline deficiency.

Since, as shown in table II, gelatin effectively prevented the accumulation of fat in the liver, and since gelatin is a relatively pure protein, it seemed logical to determine whether certain of the component amino acids of gelatin might be responsible for this effect. Results obtained when groups of 5 or 6 amino acids, in the amounts provided by 6 percent of gelatin, were included in the basal diet are presented in table V. Only one misture of amino acids was effective. This contained arginine, phenylalanine, threonine, glycine, and proline. In subsequent experiments the individual amino acids were tested and in table VI typical results are presented. From these it is evident that arginine, proline, and tryptophan were without effect, that glycine caused

Table V - Effect of Mixtures of Amino Acids on Fat Deposition in the Livers of Rats Fed 9% Casein Diets Containing Choline.

| | Rate of Gain (5 weeks) gm./wk. | Liver Fat % dry wt. |
|---|---|---|
| 9% Casein-sucrose (basal) | 15.5 ± 1.7 | 25.9 ± 2.1 |
| Basal + amino acid mix 1 | 18.3 ± 0.8 | 11.1 ± 0.8 |
| Basal + amino acid mix 2 | 19.4 ± 2.4 | 24.3 ± 1.1 |
| Basal + amino acid mix 3 | 17.6 ± 1.0 | 24.0 ± 1.8 |

Amino acid mix 1 contained: 0.56% L-arginine, HCl, 1.5% glycine, 0.22% DL-leucine, 0.24% DL-phenylalanine, 0.92% L-proline and 0.18% DL-threonine.

Table VI - Influence of Amino Acids in Diet on Deposition of Fat in Livers of Rats Receiving 9% Casein Rations Containing Choline.

| | Rate of gain (4 wks.) gm./wk. | Liver fat (dry wt). % |
|---|---|---|
| 9% Casein-sucrose (basal) | 17.7 ± 1.2 | 30.1 ± 2.8 |
| Basal + 0.56% L-arginine | 13.8 ± 0.7 | 26.2 ± 3.3 |
| Basal + 0.92% L-proline | 15.8 ± 1.3 | 28.0 ± 2.8 |
| Basal + 0.1% DL-tryptophan | 20.9 ± 1.9 | 31.4 ± 2.8 |
| Basal + 1.5% glycine | 16.4 ± 1.8 | 18.3 ± 0.9 |
| Basal + 0.18% DL-threonine | 9.7 ± 1.6 | 12.4 ± 2.0 |
| Basal + 0.18% DL-threonine + 6% gelatin | 14.5 ± 1.0 | 10.1 ± 0.5 |

some reduction in fat deposition and that DL-threonine at a level of 0.18 percent was almost as effective as the 6 percent gelatin. Since Singal and his associates, who have also studied the effects of amino acids on fat deposition in the liver (12,13), have shown that only L-threonine (the naturally occurring form) is effective in reducing the fat deposition, the amount of L-threonine required is actually about 0.1 percent. The effect of this amount of threonine is much greater than that of 1.0 percent of choline. When all of this information is considered it appears that the fat accumulation in the livers of rats fed 9 percent casein-sucrose diets results primarily from a deficiency of threonine which is overcome by the inclusion of 6 percent of gelatin in the diet.

This effect of threonine is interesting from another point of view. Since the basal casein diet was supplemented with methionine, tryptophan was limiting for growth. Only when tryptophan was provided, was a growth response obtained with either gelatin or threonine. The effect of gelatin and of threonine in reducing fat deposition, even when no growth response was observed, suggests that criteria other than growth may be important in determining the supplementary value of proteins and amino acids.

In view of this, some interrelationships among the three amino acids most limiting for growth have been studied. The results in table VII show that fat did not accumulate in the liver unless methionine (or cystine) was

Table VII - Effect of Methionine Level on Fat Deposition in the Livers of Rats Fed 9% Casein Diets Containing Choline

| | Rate of Gain (2 weeks) gm./wk. | Liver Fat % dry wt. |
|---|---|---|
| 9% Casein-sucrose | | |
| No methionine | 10.3 ± 1.4 | 16.4 ± 1.0 |
| Basal + 0.1% DL-methionine | 14.4 ± 2.1 | 24.6 ± 1.2 |
| Basal + 0.3% DL-methionine | 16.8 ± 1.1 | 26.1 ± 2.3 |
| Basal + 0.6% DL-methionine | 15.2 ± 1.0 | 23.9 ± 2.6 |
| Basal + 0.6% DL-methionine + 0.36% DL-threonine | 18.1 ± 0.5 | 14.0 ± 0.9 |

included in the basal diet (14). This general practice of supplementing low casein diets with the sulfur amino acids apparently makes tryptophan and threonine much more limiting. The addition of tryptophan either in the presence or in the absence of methionine, had little effect on fat deposition. When gelatin was included with both methionine and tryptophan growth was greatly stimulated (table VIII) and gelatin was somewhat less effective in reducing fat deposition. Apparently the marked increase in growth increased the demand for threonine for general tissue synthesis, making it somewhat less available for the control of fat deposition. However, despite this rapid rate of growth, a substantial reduction in the level of liver fat resulted from the inclusion of gelatin in the diet.

Some results with other proteins are presented in tables IX and X. When low fibrin diets were fed, the level of liver fat remained close to normal, probably because of the extremely good balance of amino acids provided by this protein. On the other hand, when albumin diets were used the results were similar to those reported for animals fed the low casein diets. Fat accumulated in the livers of young rats fed the 9 percent albumin-sucrose diet and this was reduced by the inclusion of 6 percent of gelatin in the diet. In this case the effect of gelatin could not be duplicated by threonine alone.

Table VIII - Influence of Level of Tryptophan in Diet on Deposition of Fat in Livers of Rats Receiving 9% Casein Rations Containing Choline.

| | Rate of gain (2 weeks) gm/wk | Liver fat (dry wt.) % |
|---|---|---|
| 9% Casein-sucrose (basal) | 16.3 ± 0.5 | 41.8 ± 2.6 |
| Basal + 6% gelatin | 12.3 ± 1.4 | 17.5 ± 0.7 |
| Basal + 0.05% tryptophan | 15.5 ± 1.2 | 42.8 ± 3.2 |
| Basal + 0.05% tryptophan + 6% gelatin | 24.1 ± 1.0 | 21.8 ± 1.0 |
| Basal + 0.1% tryptophan | 18.2 ± 1.8 | 38.9 ± 2.8 |
| Basal + 0.1% tryptophan + 6% gelatin | 23.0 ± 1.1 | 26.2 ± 0.8 |

Table IX - Fat Deposition in the Livers of Rats Fed Low Fibrin Diets Containing Choline.

| | Rate of gain (2 weeks) gm/wk | Liver fat (dry wt.) % |
|---|---|---|
| 6.0% fibrin sucrose | 3.2 ± 0.4 | 15.1 ± 2.1 |
| 7.5% fibrin sucrose | 5.8 ± 0.6 | 15.2 ± 1.2 |
| 9.0% fibrin sucrose | 12.8 ± 1.1 | 16.2 ± 0.9 |

Table X - Effect of Amino Acid Supplements on Fat Deposition in the Livers of Rats Fed Low Albumin Diets

| | Rate of gain (2 weeks) gm/wk | Liver fat (dry wt.) % |
|---|---|---|
| 9% albumin-sucrose (basal) | 10.0 ± 1.1 | 28.1 ± 3.3 |
| Basal + 0.36% DL-threonine | 15.1 ± 0.9 | 22.7 ± 1.3 |
| Basal + 1.5% glycine | 7.4 ± 1.3 | 16.9 ± 1.3 |
| Basal + 0.18% DL-threonine + 0.5% L-lysine-HCl | 12.8 ± 0.8 | 22.6 ± 2.2 |
| Basal + 0.36% DL-threonine + 0.5% L-lysine-HCl + 0.2% DL-valine + 0.25% L-histidine-HCl | 17.8 ± 0.6 | 13.7 ± 0.3 |

However, when a mixture of essential amino acids including threonine, histidine, valine and lysine was included, fat deposition in rats fed the low-albumin diet was reduced to the normal range. In this instance it would appear that the effectiveness of gelatin in reducing the fat deposition was due to its content of these four essential amino acids and to its content of glycine. Glycine was much more effective in rats fed the albumin diets than it was in those fed casein.

When similar experiments were carried out with young pigs the low casein basal diet did not cause fat to accumulate in the liver. The results presented in table XI show that the level of fat was extremely low even in the livers of animals fed the 9 percent casein diet and that this stiuation was not altered when the casein content of the diet was reduced to 7 percent.

Table XI - Growth and Liver Fat of Pigs Fed Low Casein Diets

| | | Rate of gain gm/wk | Liver fat (dry wt.) % |
|---|---|---|---|
| 9% casein-sucrose | 4 wks. | 3.4 ± 0.3 | 7.2 ± 1.4 |
| 9% casein-sucrose + 6% gelatin | 4 wks. | 4.6 ± 0.2 | 6.0 ± 1.4 |
| 7% casein-sucrose | 3 wks. | 1.1 ± 0.3 | 4.4 ± 0.5 |
| 7% casein-sucrose + 6% gelatin | 3 wks. | 2.7 ± 0.6 | 5.8 ± 1.1 |

Considerably higher levels of fat have been found in the livers of pigs selected at random from the production line of a packing plant so it is probable that adjustment of the experimental conditions will make it possible to study this problem experimentally in the pig. The relative life expectancy, the relative protein and amino acid requirements and possibly other factors may have to be given more attention when different species are used.

The growth results of the experiments with pigs do show that a supplement of 6 percent of gelatin stimulates the growth of young pigs fed low casein diets containing methionine and tryptophan. The gelatin supplement increased growth from 3.4 to 4.6 lbs. per week over a 4 week period.

One other point regarding the nutritive value of gelatin was also studied. Since there are reports in the literature (16) indicating that the growth of rats fed amino acid diets is reduced when part of the amino acid mixture is replaced by gelatin and since in the liver fat studies we were using gelatin as a source of amino acids, it seemed important to re-investigate this point. The results are presented in table XII. Although the growth of the amino acid

Table XII - Effect of Gelatin on Growth of Rats Fed Amino Acid Diets

| | Weekly Gain | |
|---|---|---|
| | 1st week gm | 2nd week gm |
| 18% casein + 0.3% meth. (sucrose) | 27.0 ± 2.0 | 43.0 ± 1.3 |
| 10% gelatin + amino acids ≅ 18% casein | 17.0 ± 1.5 | 27.0 ± 2.1 |
| Amino acids ≅ 18% casein | 17.0 ± 1.0 | 29.0 ± 1.8 |

group was by no means maximal it remained unchanged when part of the amino acid mixture was replaced by gelatin. This would indicate that the amino acids of gelatin are quite readily available.

As a whole this work shows that certain low protein diets cause increased fat deposition in the liver as well as reduced growth. The fatty infiltration observed is apparently the result of partial deficiencies of certain important

amino acids or of imbalances of amino acids in the diet. The exact underlying cause has not been determined but since only the naturally occurring form of threonine prevents the fatty infiltration in rats fed the low casein diets it seems that threonine must be required for the synthesis of protein. It is probable that some active protein, either an enzyme or a hormone that is important for normal liver metabolism, cannot be synthesized in sufficient quantities when threonine is low.

The reason for the effect with glycine is not yet clear and is currently being investigated. A number of other compounds in relatively large quantities (1.5 percent glycine, betaine or choline; 2.0 percent serine; 3.0 percent glutamic acid) produced similar effects. It is possible that these compounds exert a sparing effect on some essential compounds or that, in large amounts, as has been shown for choline (15), they may stimulate some metabolic process such as fat oxidation which reduces the amount of fat in the liver. The effect of these compounds appears to be distinct from that of threonine.

Chemical studies have indicated that the fat deposited in the livers of animals on the deficient diet consists largely of neutral fat. Studies on the effect of increased levels of dietary fat on liver fat deposition have indicated that the fatty infiltration does not result merely from an accumulation of dietary fat. In fact, when the level of dietary fat (corn oil) was increased from 5 percent to 20 percent, the level of fat in the liver actually decreased slightly. It appears then that either the rate of production of fat from carbohydrate is increased in the liver or that the rate of removal of fat from the liver is reduced when certain of the essential amino acids are not provided in sufficient quantities.

Although elucidation of the mechanism underlying these effects on liver fat deposition will require more extensive studies certain points of somewhat broader interest have been emphasized as a result of the observations so far. It was not apparent from the growth results that the inclusion of gelatin improved the basal diet unless the tryptophan level was also increased, nevertheless when gelatin was provided under such conditions the amount of fat deposited in the livers was distinctly reduced. These results emphasize the fact that the nutritive value of proteins, even in the growing animal, cannot always be estimated from growth results. Further, from the results obtained with such amino acids as glycine it seems probable that, in assessing the nutritive value of proteins, the nonessential amino acids may have to be given more attention.

When tryptophan and methionine were included in the low casein diets, supplementation of the diets with gelatin produced a marked growth response in both rats and pigs. Growth was similarly increased when low albumin diets supplemented with tryptophan and gelatin were fed. This effect can, of course, be predicted from an examination of the amino acid content of the proteins involved, however the effects were quite striking, and in the rats the growth response was accompanied by a decrease in fat deposition in the liver. Since cereal proteins are primarily deficient in lysine which is provided in fair quantities by gelatin, we are currently investigating the effect of gelatin as a supplement to certain cereal diets.

In summary, it is evident that, although gelatin is a nutritionally incomplete protein which cannot support life when fed as the sole source of amino acids, it can serve as a useful supplement to certain low protein diets.

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VIII

# NUTRITIONAL REQUIREMENTS OF SWINE

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The increase in our knowledge of the quantitative requirement of the pig for specific nutrients has prompted many research workers to re-examine past information with respect to protein requirement. For some years those individuals concerned with attempts to formulate balanced rations for swine have had difficulty in arriving at satisfactory values for protein. Even though some older work in this country indicated that the protein requirement of pigs of various classes was high, it was well known that under practical feeding conditions few, if any, of the pigs being raised would ever receive these levels. Indeed, according to the present production figures of protein concentrates it would be physically impossible to feed protein levels to pigs based on the old standards and have enough remaining to adequately feed poultry and other classes of livestock.

A recent development in swine nutrition which will undoubtedly have far reaching effects on the feed manufacturer as well as on the swine producer is the recognition by a number of investigators that the level of protein in growing rations can be significantly lowered.

Previous work, in this country, on the protein requirement of swine was carried out at the Pennsylvania and Illinois Experiment Stations. These results indicated that an optimum level of protein would be from 20 to 27% (1, 7, 8, 10) for the weanling pig (body weight 40 to 75 lbs.). It is interesting that the nitrogen balance method indicated a protein requirement of around 27%, while growth data gave reasonably good results at the 20% protein level. However, Keith and Miller (7) further found that when pigs were fed on rape pasture, protein levels of 9 and 12% gave satisfactory growth. These latter results indicate that the rape pasture was providing other essential nutrients, such as vitamins, which stimulated appetite because the pigs fed the 9% protein ration consumed more dry feed than did a similar group fed in a dry lot.

Canadian work on levels of protein for swine by Crampton and Ashton (3) published in 1942 was not in agreement with work in this country. The results of these investigations indicated that pigs from 30 to 100 lbs. body weight would gain normally on rations containing from 15 to 18% protein.

The English work on the protein requirement of growing pigs by Woodman and Evans (12, 13, 14, 15, 16) beginning in 1939, has apparently escaped the attention of American workers. In a series of detailed studies these workers produced convincing evidence to the effect that the protein requirement of the young growing pig was approximately 15% of the diet.

In comparing the experimental rations used by both the English and Canadian workers with those of the American investigators, it should be noted that while the grains used by the former were barley and wheat, the latter workers used corn almost exclusively as the grain component of the ration. The greater nutritional adequacy of the rations used by the English and Canadian workers undoubtedly explains the superior results with lower protein levels.

The recent awakening of interest by many investigators in the level of protein in swine feeding was stimulated by the very extensive research effort in the feeding of various antibiotics to swine. In addition, since the synthetic vitamins have been available in quantity for the past five years, it is now possible to study vitamin and protein effects independently. Thus, in 1950, Cunha et al. (4), working with young growing pigs, fed rations varying in protein content from 12.2% to 19.6% with and without an APF (Lederle) concentrate. In each case the addition of the APF increased the rate of gain, and the 17.9% protein ration with APF was equal to the 19.6% protein ration with APF. These same workers found that pigs on a 19.6% protein ration without the addition of supplementary B vitamins and without APF did not gain as well as pigs on the 12.2% protein ration supplemented with B vitamins and APF.

The fact that pigs grow very well on extremely low protein rations has been shown by Geurin et al. (5). In this case the ration used was semi-purified and contained dried skim milk as the source of protein. The level of protein in this ration was only 11%.

Catron et al. (2) have indicated that the feeding of very low protein rations resulted in good growth. In this experiment protein levels were changed at three weight periods, namely: initial to 75 lbs., 75 lbs. to 150 lbs., and 150 lbs. to 200 lbs. Four initial protein levels were used: 20%, 18%, 16%, and 14%, and each of these were reduced by 3% when the pigs passed into successive weight classifications. The initial average weight of the experimental lots varied from 29.9 to 37.5 lbs. The results very clearly indicated that weanling pigs fed the 16% protein ration grew just as well as comparable groups fed the 18% or 20% protein rations. The authors concluded that in the absence of an antibiotic the 16-13-10% combination supplied adequate protein for the pigs' need during the growth period studied.

Hoefer et al. (6) have found that low protein rations will perform in a very satisfactory manner. In this trial a corn-soybean oil meal ration was used which contained fish solubles, minerals and vitamin fortification. The results of this experiment are shown in table 1. It was found that the average daily gain of the pigs fed the 15% protein ration (reduced to 12% at 100 lbs.) was equal to the average daily gain of pigs fed the 18% protein ration (reduced to 15% at 100 lbs.). In these trials the antibiotic used did not seem to have any effect on the protein requirement.

Thus, the previously cited results together with a very large amount of excellent data reported by a number of swine investigators in this country all point to the fact that protein levels in a modern swine ration can be reduced very significantly with no adverse effect as far as growth in concerned.

The implications of the above work for the feed manufacturer and swine producer are many and varied. First of all it is well known that an animal's requirement for protein changes with each growth increment, and thus, the protein requirement of the baby pig is higher than at any other time during the growing period. Furthermore, since the widely used term "weanling pig" is certainly a very ambiguous one when used to denote body weight, it becomes even more important to determine accurately the relationship of protein level to weight in the young pig. For example, in the case of the recent experimental work on level of protein carried out by the fore-mentioned investigators, average starting group weights varied from 25 to 45 lbs. It is thus reasonable to assume that the protein requirement of the 25 lb. pig is higher than that of a 45 lb. animal. To date, the best information available on the weaning weight of pigs produced in this country lies somewhere between 25 and 30 lbs. body weight.

The importance of body weight and of level of protein can best be shown by the data presented in table 2. The data shown are individual lots taken from two trials carried out at the Michigan Experiment Station during 1951. Both trials were carried out at approximately the same time, in the same barn, and on the same feed ingredients. The rations used were of the corn-soybean oil

Table I - The Effect of Terramycin on the Growth of Pigs Fed Different Levels of Protein. (Two lots of 10 pigs per lot on each treatment)

| Weight period, lbs. | 25-100 | | | |
|---|---|---|---|---|
| Protein level, % | 18 | | 15 | |
| Terramycin added, mg./lb. | 0 | 5 | 0 | 5 |
| Average daily gain, lbs. | 1.05 | 1.42 | 1.04 | 1.45 |
| Feed per lb. gain, lbs. | 3.00 | 2.65 | 3.26 | 2.83 |
| Weight period, lbs. | 100-200 | | | |
| Protein level, % | 15 | | 12 | |
| Terramycin added, mg./lb. | 0 | 5 | 0 | 5 |
| Average daily gain, lbs. | 1.73 | 1.86 | 1.82 | 1.88 |
| Feed per lb. gain, lbs. | 4.09 | 3.93 | 4.01 | 4.00 |
| Weight period, lbs. | 25-200 | | | |
| Protein level, % | 18-15 | | 15-12 | |
| Terramycin added, mg./lb. | 0 | 5 | 0 | 5 |
| Average daily gain, lbs. | 1.35 | 1.63 | 1.32 | 1.65 |
| Feed per lb. gain, lbs. | 3.62 | 3.35 | 3.63 | 3.45 |

meal type containing, in addition, 2% condensed fish solubles and were fortified with vitamins. All of the animals were of the same breed. Since the pigs were weighed every two weeks, it was possible to calculate the average daily gains for each two week interval. The results clearly indicate that in Experiment A the feeding of a well fortified ration containing only 14% crude protein resulted in very subnormal growth in the case of pigs averaging only 21 lbs. in weight. However, the 18% protein ration gave normal average daily gains. On the other hand, it can be seen from Experiment B that the feeding of the 15% crude protein ration to pigs averaging 25 lbs. body weight resulted in daily gains which were equal to the group fed the 18% protein ration. Thus, caution should be exercised in any recommendation concerning the feeding of low protein rations for young weaned pigs, since under field conditions low protein rations would almost certainly be fed to pigs weighing around 20 lbs.

In attempting to evaluate the factors responsible for the success of lower protein rations, it is apparent that the nutritional developments of the past twenty years have had a part in making this possible. The development and continual improvements in our available protein concentrates, particularly soybean oil meal, are very important factors. The fact that with a purified diet containing dried skim milk or casein, excellent growth is obtained on levels of protein of the order of 10 to 12% with the 30 lb. pig, certainly indicates the importance of the supplemental protein source. The importance of the addition of high quality protein concentrates to corn in low protein rations cannot be overemphasized. For example, in a ration containing 14% crude protein, with corn as the grain source, the contribution of corn protein to the total protein in the ration amounts to almost 50%. Since corn protein is low in certain essential amino acids, the protein concentrate used must be of such a quality that a relatively small amonut will supplement the corn protein to provide an available amino acid balance in the total ration.

In the recent work with low protein rations, B vitamin fortification was used extensively. The value of these additions to many rations made up of natural feed ingredients has been demonstrated by many workers. However,



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Table II - Growth Response of Pigs Per Two Week Interval on Different Levels of Protein (Ten pigs per lot)

| Expt. no. | Protein level | Av. initial wt. | Average daily gain for successive two weeks of trial | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 6 | 8 | 10 | |
| | % | lbs. | lbs. | lbs. | lbs. | lbs. | lbs. | |
| A[1] | 14.0 | 21<br>18-25[3] | 0.38 | 0.58 | 0.87 | 1.04 | 1.14 | (0.81)[4] |
| A[1] | 17.8 | 21<br>18-24 | 0.78 | 1.04 | 1.16 | 1.28 | 1.42 | (1.13)[4] |
| B[2] | 15.0 | 25<br>20-28 | 0.73 | 0.88 | 1.33 | 1.31 | 1.66 | (1.17)[4] |
| B[2] | 18.0 | 25<br>20-28 | 0.71 | 1.00 | 1.22 | 1.33 | 1.60 | (1.09)[4] |

1. Data taken from trial published by Luecke et al. (9)
2. Data taken from trial published by Hoefer et al. (6)
3. Range of initial weights.
4. Average daily gains for the entire 10 week period.

since protein concentrates, in general, are good sources of the B factors, the less protein concentrate in the total ration the lower would be the vitamin content of the finished feed. Additional evidence on the value of B vitamin supplements in low protein swine rations was presented by Wahlstrom (11).

Inasmuch as most commercial swine feeds are sold to the producer as supplements, the field use of low protein rations presents a very practical problem to the feed industry. The modern swine supplement is not merely a protein supplement; it must contain all of the ingredients necessary to balance grain, particularly corn. Thus, it becomes a protein-vitamin-mineral supplement with each one of the factors as important as the other. An example of what might happen if the producer, using a commercial swine supplement, should reduce the protein content of his ration from 18% to 14% is shown in tables 3 and 4. Table 3 shows the average and range values for the niacin,

Table III – Niacin, Riboflavin and Pantothenic Acid Content of Forty-five Commerical Swine Supplements[1]

| | Protein | Niacin | Riboflavin | Pantothenic acid |
|---|---|---|---|---|
| | % | mg./lb. | mg./lb. | mg./lb. |
| Average | 37.8 | 21.02 | 2.84 | 8.08 |
| Range | 30-40 | 10.72-72.87 | 1.52-9.61 | 5.18-24.57 |

1. Samples supplied by the Michigan State Department of Agriculture in 1951 and were all assayed microbiologically.

riboflavin and pantothenic acid content of forty-five commerical swine supplements sold in Michigan. From this is obtained the vitamin content of the "average" supplement. With this as a basis and using average protein and vitamin values for corn, it is possible to calculate the niacin, riboflavin and pantothenic acid contents of rations made up of different proportions of corn and "average" supplement. This is done in table 4 for two rations, one calculated to contain 18%, the other 14% protein. Table 3 shows very significant reductions in the riboflavin and pantothenic acid content of the finished feed made up to contain 14% protein when compared with the 18% protein ration. The reduction in niacin content is less extreme.

Table IV - Niacin, Riboflavin and Pantothenic Acid Contents of Rations Containing the Average Commercial Supplement and Corn at Two Different Protein Levels[1]

| | Niacin | Riboflavin | Pantothenic acid |
|---|---|---|---|
| | mg./lb. | mg./lb. | mg./lb. |
| For 18% protein ration[2] | 13.64 | 1.27 | 4.33 |
| For 14% protein ration[3] | 12.09 | 0.93 | 3.53 |
| Percent reduction in vitamin values of 14% over 18% protein ration | 11.5 | 26.4 | 18.4 |

1. Values for corn taken from Feed Ingredient Analysis Table, The Feed Bag Red Book, 1952.
2. Ration composed of 32% supplement and 68% corn.
3. Ration composed of 18% supplement and 82% corn.

This serves to illustrate that a supplement formulated to balance corn for an 18% protein feed might prove entirely inadequate when used to formulate a 14% protein ration. It is not the author's intention to overemphasize niacin, riboflavin and pantothenic acid, but since quantitative data was available a reasonably accurate example could be drawn. Certainly, reduction of mineral content would be equally if not more important. It can be seen from the footnote in table 3 that it required 44% less supplement to make up a 14% protein ration as an 18% protein ration. Thus, since grains are very poor sources of calcium, the supplement must supply almost all of this mineral and a reduction in the amount of supplement used would reduce the calcium content of the resulting ration by very significant amounts.

Thus, the sum total of experimental results indicates that it will be practical to recommend lower protein rations for growing swine. The resulting savings in the total cost of pork production will be significant provided that sufficient attention is paid to the important factors other than protein in the formulation of adequate swine rations.

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IX

# USE OF ANIMAL FATS IN FEEDS

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Recent studies have emphasized the attributes of added fats in feeds for dogs, poultry, swine, sheep, and cattle. While the nutritional studies have revealed an increase in feed efficiency associated with the addition of animal fats, other advantages including control of dustiness in the ration and mixing operations and other physical factors have been of great importance in the application of these findings to commercial practice.

The fat content of practical-type rations has progressively decreased in the past several years with more effective extraction of the fats from the feed ingredients. This gradual decline in fat content is perhaps overlooked as an important factor in the very favorable results obtained when 2-8% animal fats are added to such rations.

In evaluating the role of added animal fats in feeds, the primary information needed included nutritional tests with these fats added to practical-type diets, and chemical tests on the stability of these fats and of vitamin A when added to feeds. Approximately four years ago, such basic nutritional experiments were initiated in our laboratory in which the effect of adding 2-8% animal fats, stabilized with appropriate antioxidants, was studied with dogs and chicks. Choice, white grease, a so-called inedible pork fat, was used and added to rations composed of ingredients commonly used for commercial type rations. The performance of dogs and chicks fed diets containing added animal fats was excellent.

Other studies conducted at the University of Wisconsin, University of Nebraska, University of Florida, Purdue University, Texas A & M College, Oklahoma A & M College, and other laboratories also indicate that animal fats can be used successfully in rations for poultry, beef cattle, swine, and sheep.

Insuring the stability of the added fat also is important. In our experience, feeds containing animal fats stabilized with antioxidants have not become rancid after room temperature storage of the feed for 1 year. Further, the stability of vitamin A, added as fish liver oil, was enhanced by the addition of stabilized animal fats to feeds stored for a similar period.

These studies provided the necessary ground work in establishing the value of animal fats in feeds. Many feed companies are now adding stabilized animal fats to their commercial feeds. While outlining the economic importance of this development to the meat packing and rendering industries is beyond the scope of this paper, it is pertinent to point out that if only 1 cent per pound of the increased price for animal fats that has occurred is attributed to this development, this amounts to a value of $ 25,000,000 per year to the industry.

Since all our previous nutritional studies were conducted with choice, white grease, it was important to extend our studies to different grades of animal fats. These fats differ primarily in the amounts of free fatty acids

present, and in the titer (solidification temperature of the fatty acids prepared by saponification of the fat). Five different grades of feed grade fats were obtained and stabilized with an antioxidant mixture containing butylated hydroxyanisole. The analytical data obtained on these fats by our Service Laboratory and on fatty acids prepared from choice, white grease are presented in Table 1.

Table I - Analytical Data on Different Animal Fats

| Animal Fat | Titer | Free Fatty Acids | A.O.M. Stability* | |
|---|---|---|---|---|
| | | | Before | After |
| | °C | % | hrs. | hrs. |
| Choice white grease | 39.3 | 2.9 | 10 1/2 | 100 |
| Yellow grease | 40.5 | 17.1 | 8 1/2 | 107 1/2 |
| Brown grease | 42.4 | 58.8 | 10 | 47 |
| Prime tallow | 41.6 | 3.2 | 9 | 97 1/2 |
| No. 2 tallow | 42.3 | 30.5 | 11 1/2 | 96 |
| Fatty acids prepared from choice white grease | | 100 | 6 | 52 1/2 |

* Before and after stabilization with 0.02% butylated hydroxyanisole, 0.01% citric acid, and 0.005% propyl gallate.

Experiments were then run with chicks fed a basal ration similar to that used in previous studies (Table 2), or this ration plus 3 or 6% of each of the animal fats added at the expense of the entire ration. A group that was fed 3% fatty acids was included to determine if the free fatty acid content of

Table II - Basal Rations for Chicks*

| Ingredient | % |
|---|---|
| Yellow corn | 65.2 |
| Soybean oil meal | 16.0 |
| Meat and bone scrap | 8.0 |
| Fish meal | 3.0 |
| Blood meal | 2.0 |
| Corn gluten meal | 2.0 |
| Alfalfa meal | 2.0 |
| Butyl fermentation solubles | 1.0 |
| Fish liver oil | 0.3 |
| Iodized salt | 0.5 |

* Aureomycin, choline, niacin, and manganese supplements also were included.

animal fats might be related to the performance of animals. This was particularly important since we could see no reason why fats high in free fatty acids, as a result of hydrolytic cleavage of the glycerides and not from oxidative rancidity, would not be nutritionally satisfactory.

Duplicate experiments are being conducted and the results after the chicks have been on experiment for 6 weeks are presented in Tables 3 and 4. It can be seen from these data that no major differences in the rate of gain occurred regardless of the level or kind of fat added. Since the second

Table III - Performance of Chicks Fed Different Grades of Fats (Weight at 6 weeks)

| Supplement to Basal Ration | Exp. I. | Exp. II. |
|---|---|---|
| | g. | g. |
| None | 649 | 771 |
| 3% Choice grease | 669 | 742 |
| 3% Yellow grease | 690 | 741 |
| 3% Brown grease | 643 | 819 |
| 3% Prime tallow | 648 | 781 |
| 3% No. 2 tallow | 633 | 792 |
| 3% Fatty acids prepared from choice grease | 671 | 788 |

Table IV - Performance of Chicks Fed Different Grades of Fats (Weight at 6 weeks)

| Supplement to Basal Ration | Exp. I. | Exp. II. |
|---|---|---|
| | g. | g. |
| None | 649 | 771 |
| 6% Choice grease | 643 | 748 |
| 6% Yellow grease | 672 | 766 |
| 6% Brown grease | 657 | 777 |
| 6% Prime tallow | 643 | 790 |
| 6% No. 2 tallow | 652 | 731 |

experiment is not yet complete, final evaluations of the rates of gain, feed efficiency, etc., cannot be made at the present time. From the data to date, it is evident that the growth performance is not correlated with the titer within the range studied (39.3-42.4°C.) nor with the free fatty acids in the fat supplements (2.9-100%). White Rock male chicks were used in these studies (approx. 25 chicks per group), and it is of interest that the chicks used in the second experiment are gaining at a more rapid rate than those from the first experiment. It should be emphasized that these fats were not rancid and were effectively stabilized (see Table 1). Further, taste panel evaluations of birds fed the basal ration or basal plus choice, white grease or brown grease showed no differences in quality between groups. Further work on this aspect also will be carried out.

Other studies being conducted by Drs. Wilder and Dugan and associates on the addition of antioxidants during rendering will provide valuable data on the importance of stabilizing the residual fat in meat and bone scrap as well as in the rendered fat.

The value of animal fats in controlling dustiness in dehydrated alfalfa has raised important questions. Extensive research on the effectiveness of antioxidant-treated animal fats in increasing the stability of the carotene contributed by alfalfa and other ingredients in the ration and in increasing the absorption and utilization of fat soluble vitamins by the animal will be of much value.

Further studies will be conducted in our laboratories on the stability of vitamin A and carotene (in alfalfa) when mixed in feeds that contain animal fats that have been either stabilized or not stabilized with antioxidants. The stability of vitamin A will be followed chemically and by bio-assay and information on the effect of antioxidant-treated fats on the utilization of

vitamin A and carotene also will be determined.

It is quite likely that added animal fats in the ration may have a role in certain syndromes observed in chicks and other animals fed practical rations. Thus, the hemorrhagic condition observed in chicks, which responds at least in part to vitamin K additions to the ration, may be improved by adding animal fats to the ration since an increase in the fat content of the ration may enhance the absorption and utilization of vitamin K and other fat soluble vitamins contributed by the ration. Experiments along these lines will be of great interest and importance.

The nutritional value of stabilized animal fats in practical rations, therefore, may extend beyond increased food utilization and palatability to enhanced fat soluble vitamin stability in the ration and increased absorption and utilization of these nutrients by the animal. It is evident, therefore, that while much progress has been made recently in understanding the role and importance of animal fats in nutrition, many interesting and vital problems on fat digestion, absorption, utilization, and stability of other nutrients await further study.

X

# TURBO CHILL IN THE MEAT INDUSTRY

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Chicago, Illinois

In many industries there are requirements for cooling, heating or drying of the product. When these industries were young and the profit dollar less elusive, these processes were unhurried and carried on by the action of heat and temperature differences, chiefly by radiation and natural convection. Space occupied and time required were not of the essence. Now most of this work, under the pressure of present day economics, is a job for a fan. The fan with its auxiliary finned coils and air washers provides controllable quantity, temperature and humidity.

In the packing industry fans have not been too well received. For the hot meat cooler and the holding rooms the brine spray with natural gravity flow is still in high favor. Probably the chief reason for the persistence of an old method, aside from an understandable inertia in connection with a costly and perishable product, can be found in the limitations of the condition of the air delivered from the conventional fan, coil and washer. With such a system the very limit of humidity is 100% and even this limit is difficult to attain. Entering a warmer room such air would have a drying capacity and with high velocity would produce large shrink and even windburn. Turbo Chill with its super-saturation avoids these difficulties.

## 1. METHOD AND APPARTUS

The Turbo Chill method of cooling and holding meat provides, first and primarily, super-saturated cold air; second, a rapid and controllable air change; and third, close thermostatic adjustment.

The super-saturation in the cold air entering the cooler chamber reduces the shrink. The rapid circulation of air cools the outer surface of the meat quickly, preventing the formation of slime despite the high humidity. Also the high air velocities give faster cooling. Even with these velocities the super-saturation prevents windburn. As the air flow is fan actuated, exact and quick acting thermostatic control becomes part of the system.

The apparatus consists of a fan type compressor, a cooling coil, an air turbine and a motor to supply losses. The compressor draws from the room and increases the pressure and temperature of the air. The cooling coil removes heat and reduces the temperature of this high pressure air but not to the dew point. The turbine then converts the volume flow and the high pressure into power with the resultant further heat drop to the final temperature. The time interval of flow through the turbine is too short to permit the complete heat transfer for condensation and the air from the turbine exhaust re-enters the room in a super-saturated metastable state. There is no change in specific humidity during the flow through the fan, coil and turbine.

## 2. THERMODYNAMICS

For practical engineering purposes we can approximate closely enough the performance of fan type compressors and turbines by the simplifying assumption of adiabatic processes.

When we compress air we increase its pressure and temperature. The temperature rise TR will be given by the simple formula

$$TR = \frac{42.4 \times \text{Horse Power Input}}{\text{Weight of Air per min.} \times \text{Specific Heat of Air}}$$

Similarly for the turbine with a temperature drop TD

$$TD = \frac{42.4 \times \text{Horse Power Output}}{\text{Weight of Air per min.} \times \text{Specific Heat of Air}}$$

The advantage of using these formulas is that the final result can be calculated from air volumes and temperatures measured in the unsaturated flow to the turbine and the readings of accurate electrical instruments. Uncoded procedure is avoided.

The horse power output of the turbine helps drive the fan but this returned power will be less than the input to the compressor by the inefficiency losses in the two machines plus a loss due to friction through the coil and the reduction in volume due to cooling. These losses must be supplied by the motor, and the heat equivalent of the motor power removed by the coil refrigeration. If the motor is in the refrigerated space we must allow for the motor input; if the motor is outside the space we must allow for the motor output.

The necessary tons of refrigeration will then equal the useful refrigeration for the cooler plus the motor power equivalent. For identical cooler refrigeration the kind of system used has no effect on the demand. The only possible difference is in the motor power used.

For this identical cooler action we can offer the reduced shrink. While this has been the chief aim, the fact that we are not limited by natural convection makes possible additional objectives. We can make use of larger refrigeration capacity. Thereby we can attain the same cooling in a shorter time or lower meat temperatures in the same time. Further we can control cooler temperatures, keeping down the rise during the initial hot loading period and preventing too low temperatures at the end of the cooling.

## 3. SUPER-SATURATION EFFECT

Super-saturation gives a condition that can be secured in no other way. With a given temperature the vapor pressure with super-saturation is higher than the vapor pressure at a water surface, for example, the wet surface of the meat. The meat surface then can be warmer than the air and still have no vapor pressure difference. Some heat can thus be transferred as sensible heat without proportionate evaporation from the meat.

Now super-saturation without condensation can exist and, in the absence of finite disturbance, can persist in the metastable state. It has been most interesting to observe this phenomenon in actual operation. Much of the time the turbine exhaust is clear, demonstrating metastability. Then at times a faint bluish haze appears—the diffraction of light by molecular sized particles. At still other times the definite smoke-like fog, which pervades the room, extends to within a foot or two of the turbine exhaust. This fog state is the stable one. Dust or other nuclei in the air provide the finite disturbance to bring about the stable state. The final size of the drops is small enough that super-saturation subsists. But disregarding this capillary pressure, we can calculate the temperature of thermal equilibrium from a heat balance with the latent heat

of vapor condensed equal to the increase in sensible heat of the mixture of air, saturated vapor and water. This state is probably not attained as there is a continual supply of super-saturation as vapor and fog to the chamber.

Consequently at all times we have a vapor pressure in the air higher than the saturation pressure at the existing temperature. There is thus always a vapor block to reduce shrink.

## 4. COMMERCIAL ECONOMICS

As almost always is the case in the useful application of a scientific principle obeisance must be made to economic considerations. Engineering success is vitally dependent on the soundness of the judgment used in making the compromises between what we should like to do and what it is profitable to do.

Cooling meat is a complicated procedure. Fortunately, we realized in the very beginning of our work that we were dealing with an art as well as a science. We therefore consulted all available experts in the local packing industries that we could interest in our project. They were all friendly and helpful. We, of course, encountered the skepticism that is always a part of a research development, but even the skepticism helps. A research man must accept the fact that properly enough the burden of proof is on anyone who is trying to do something new. However, the researcher is only human and is entitled to at least one comeback. Our decisions would have been simpler and our work easier if the experts hadn't differed one from another and sometimes, it seemed to us, one from all the others.

We tried to pick out the fundamentals such as:

1. Preservation of the bloom.
2. A firm carcass.
3. A dry surface.
4. Fast cooling.
5. Low shrink.

In our laboratory pilot plant we found we could meet these requirements with a super-saturation of approximately one-sixth with the metastable state temperature of the air leaving the turbine of 30° and the stable fog state at 32°. The air flow was 620 cfm per ton of meat as a maximum.

Under these conditions we had cool carcasses with proper bloom that were firm and dry. The time required was less than with conventional methods. The shrink in the cooler was lower than claimed for any of the successful present day systems. For these results the cost of apparatus and the charges for extra power and refrigeration is negligible as compared with the money value of the saving in shrink.

Although we have found that practices and results vary widely in the different packing companies we have yet to find one where Turbo Chill cooling would not pay a handsome return. The variation in practice in the industry would impress any investigator as somewhat mystifying. No doubt there are reasons other than what appear to be merely individual preferences. But one plant wants to cool to 45° in the deep round; while another standardizes on 58°. Some use cooling needles inserted into the rounds. The loading of the coolers varies almost 100%. Some plants admit serious losses from sour rounds, others claim insignificant trouble and still others complete freedom from sours.

It is obvious that, for the present, each prospective job will require its own analysis. The temperature specifications alone would make a great difference.

## 5. PERFORMANCE

Turbo Chill has been subjected to extensive investigation. The laboratory equipment has a capacity of 16 sides and was frequently loaded with over three tons of meat. Various loadings were tested down to a minimum of four sides with a total weight of 1,650 lbs. We tested beef, heavy and light, and several loads of hogs. The work extended throughout about 18 months. We were gratified to find cooler shrinks in all cases of the order of 1%. These figures have been confirmed in the large commercial cooler. In this latter case we made no selection but took animals of all grades as they came in regular production. We found that cutters and canners shrink more than high grade steers. A good layer of fat is highly conducive to low shrink. The average shrink in the cooler for all grades has been 1.2% with the high grade steers sometimes as low as 0.9 and seldom above 1.1.

We discovered early in our work that packing house operations do not follow time schedules to the minute. Some times we would get our sides a few minutes after they left the hot beef scales and at other times it would be half an hour. In a half-hour the wash water would have dripped off and there was, we believe, also some evaporative shrink. The action in our coolers, small and large, has been repeated and consistent. With a half-hour drip time, the loss before entering the cooler was approximately 0.4% which added to 1.0% in the cooler gives a figure of 1.4%. However, when we got the sides quickly enough so that the weight entering our cooler was substantially the same as the weight on the kill floor scales, the total loss of drip and cooling was only slightly greater than the previously mentioned 1.2% loss for cooling alone.

The indication is that the actual wash water drip is a small amount and that during a half-hour delay there is an excess and unnecessary shrink at room temperature. In a test with 120 animals, good and choice, in our cooler, in which test there was a negligible loss of so-called drip, the total shrink averaged 1.23% from kill floor to shipping weight. These cattle were cooled in twenty-four hours and held before shipment for another twenty-four hour period. These identical carcases with their low shrink were shipped to Florida to test a criticism that low cooler shrink would be offset by greater loss in the cars. The criticism was disproved. The losses were the same for both methods of cooling.

This particular test with a cooler full of animals was undertaken to check the many similar single instances we encountered in our regular production tests. The animals should be put in the cooler, any cooler, as quickly as possible. Incidentally, this result suggests the possibility of doing the hot cooling on a chain belt in a tunnel. The present methods of cooling move the coolant air slowly by convection. With the 80 to 90% relative humidity in a brine spray cooler a high air velocity, even if available, would produce windburn. A fan blowing this air would make matters even worse as the power taken by the fan would increase the air temperature and lower the already too low, relative humidity.

With Turbo Chill's super-saturation high velocity is definitely advantageous. In our test work we have had velocities up to 5,000 fpm blowing directly on the meat with no windburn, no increase in shrink and with faster cooling. Additional pressure can be provided to blow the air through a tunnel. Even counterflow could be utilized. Turbo Chill can offer the possibility of entirely new planning for meat refrigeration. Turbo Chill can make major savings in space, time and shrink and can increase profits.

## 6. APPENDIX

Curves of actual test performances are attached.

Curve No. 1 shows the deep round temperature time curve in the laboratory cooler for average weight carcasses of 500 to 600 lbs. This test was made with high velocities and turbulence. The cooling chamber was uncomfortable to work in. The curve is presented to show possible results. In tunnel cooling the times shown could be further reduced and men would not have to work in the space.

Curve No. 2 is the same curve for the commercial cooler with velocities and turbulence cut down to give good working conditions. The times required in a neighboring brine spray cooler were 26% longer.

Curve No. 3 gives the useful refrigeration co-efficient in B.T.U.s per lb. of meat during a 48 hour run. Also the air temperatures in the cooler. The curves are presented both for brine spray and Turbo Chill. Average deep round temperatures in the two coolers are given for the heaviest carcass. On this sheet is shown the value of Turbo Chill's adaptability to heat load. At the time of loading the heat load was high. The curves show that the Turbo could and did supply excess refrigeration to suit. The room temperature was kept down to a safe 38 1/2°. With the brine spray the temperature went to 47° and this spray cooler was specially provided with 35% extra nozzle capacity.

It has been suggested that the terms used in the discussion be defined in this appendix.

Specific heat of air. - The amount of heat required to increase the temperature of one lb. of water 1°F is a B.T.U. or British Thermal unit. The amount of heat required to raise one lb. of air 1°F is only 0.172 B.T.U.s if the air is confined to a constant volume. But, if the air is free to expand with heat, extra heat is required to provide the expansion energy and 0.24 B.T.U.s are needed for the 1° rise. This latter is usually the case in engineering and is called, not too exactly, the specific heat at constant pressure.

$$Cp = 0.24$$

Adiabatic. - Of or pertaining to a process in which there is no transfer of heat as heat.

Specific humidity. - The pounds of water vapor per pound of dry air.

Relative humidity. - The ratio in percent of the actual vapor pressure to the saturation pressure of vapor or steam at the existing temperature.

Reduced to values per 100,000 lbs. of meat, twenty-four hour calculations, based on test results, give the following significant figures for recirculation when constant temperatures are reached.

| | |
|---|---|
| Poundage | 100,000 |
| Air CFM | 32,000 |
| " Lbs. | 2,510 |
| Barometer Chicago | 29.2 |
| Fan Pressure | 17.5" Water |
| Return Air Temp. | 34° |
| Return Air Rel. Humidity | 100% |
| Fan Efficiency | 88% |
| Temp. After Fan | 41.7° |
| Press. Loss Coil | 1" |
| Temp. Drop Coil | 6.07° |

| | |
|---|---|
| Turbine Efficiency | 85% |
| Temp. Entering Turbine | 35° |
| Temp. Drop Turbine | 4.81° |
| Temp. Leaving Turbine | 30.19° |
| Horse Power Motor—Out | 32.3 |
| Specific Humidity | |
| 34° saturated | 0.0042 |
| 30.2° | 0.0036 |
| Super-saturation | 0.0006 |

B.T.U. Balance

| | | |
|---|---|---|
| Coil | 2510 x 6.07 x 0.24 = | 3660 |
| Cooler | 2510 x 3.81 x 0.24 = 2290 | |
| Motor | 32.3 x 42.4 = 1370 | |

Temperature differences are greater before the above steady state is established. During the early part of the run most of the shrink occurs.

| | | |
|---|---|---|
| Latent Heat | 1.1% shrink | |
| | 100,000 x 0.011 x 1,062 = 1,170,000 | |
| Sensible Heat | | |
| | 2,290 x 1,440 | = 3,300,000 |
| Total B.T.U.s in 24 hours | | = 4,470,000 |

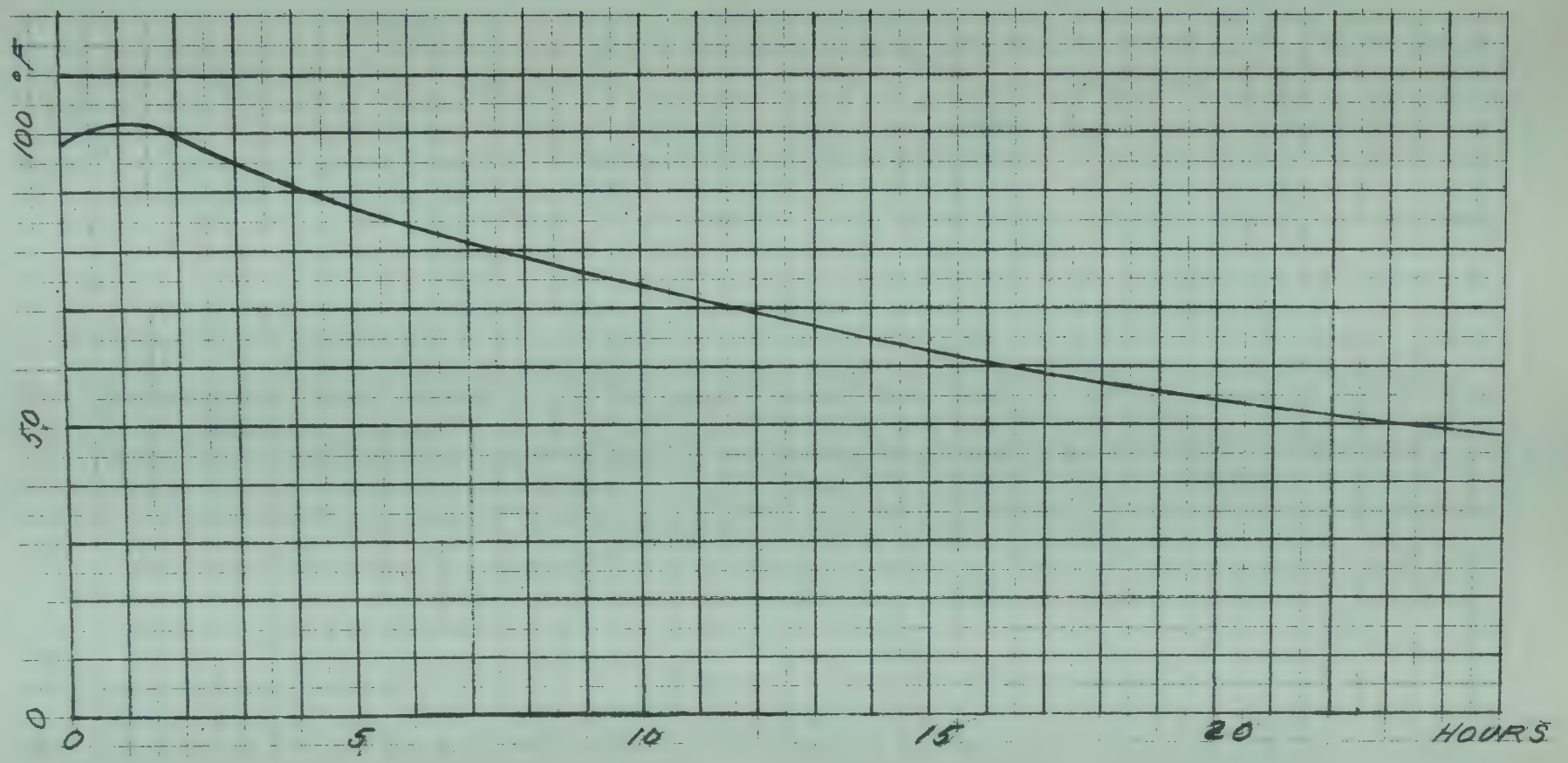


Figure 1 - Turbo Chill
Curve # 2
Cooling-Low Velocity

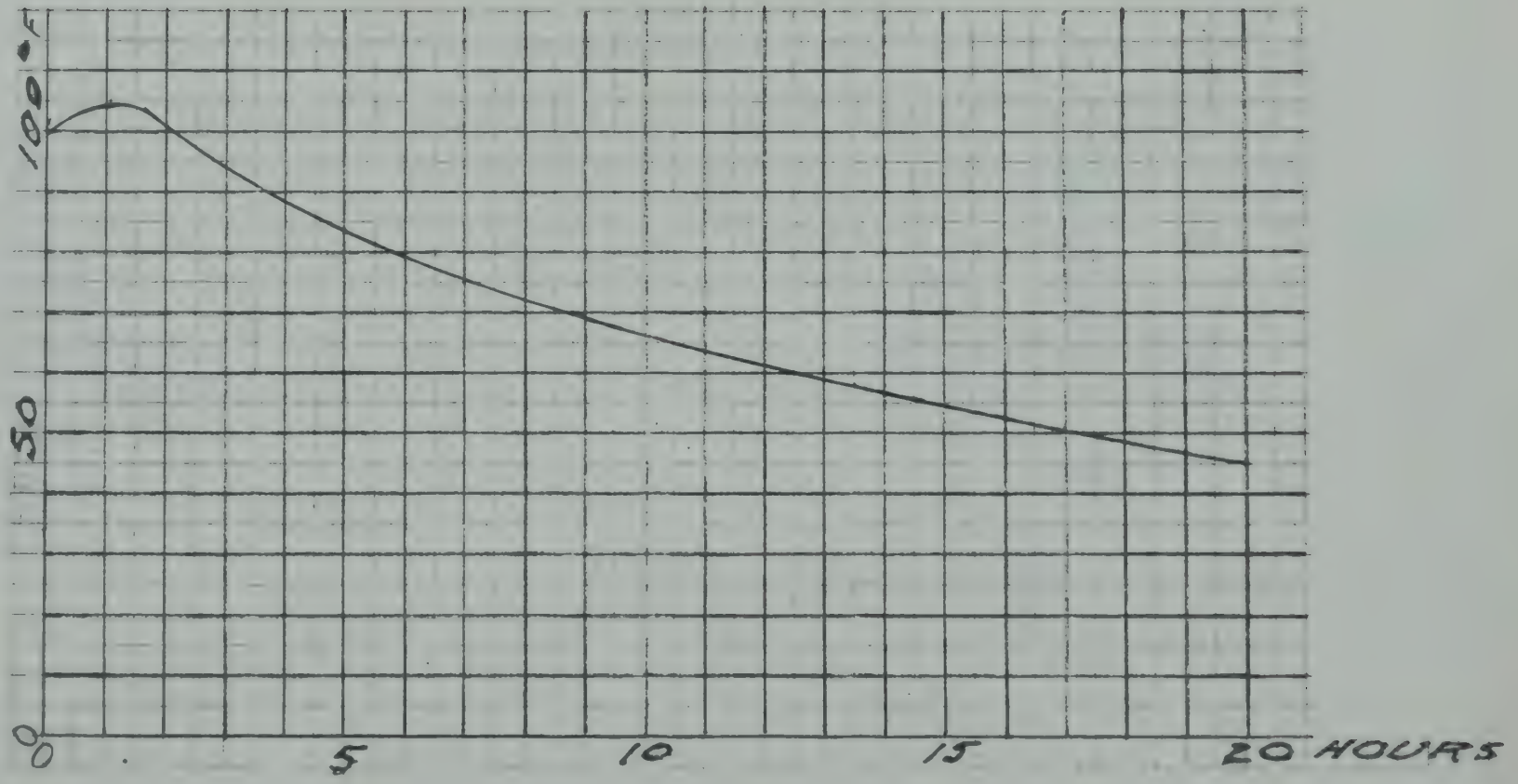


Figure 2 - Turbo Chill
Curve # 1
Cooling-Turbulent

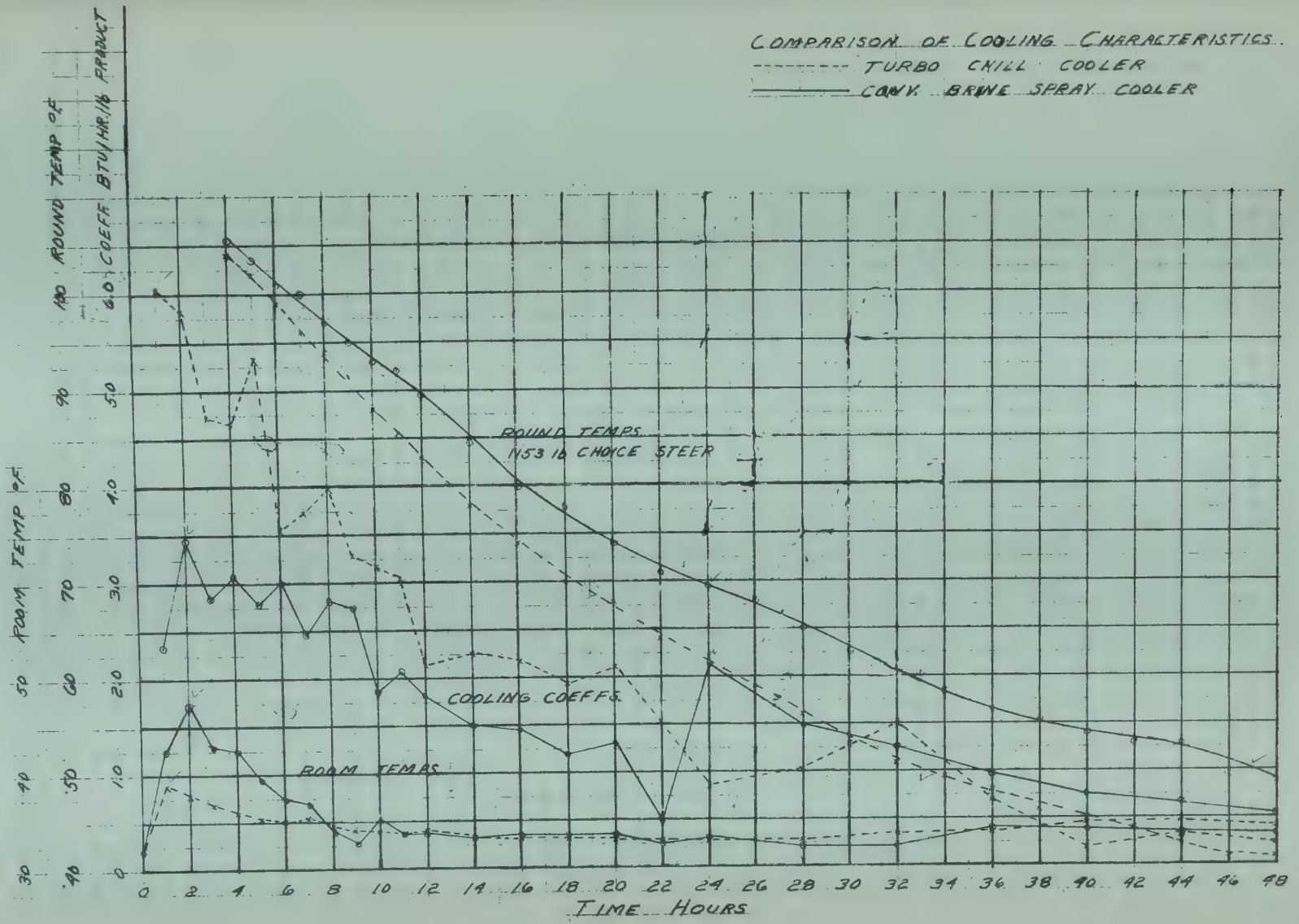
COMPARISON OF COOLING CHARACTERISTICS
TURBO CHILL COOLER
CONV. BRINE SPRAY COOLER
ROUND TEMP °F
COEFF BTU/HR/lb PRODUCT
ROOM TEMP °F
ROUND TEMPS
1153 lb CHOICE STEER
COOLING COEFFS.
ROOM TEMPS
TIME HOURS
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48

**Figure 3**

XI

# LIVESTOCK AND MEAT RESEARCH

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Our program of livestock and meat research has as its major objectives the improvement of efficiency of livestock production and the adjustment of kinds and qualities of meat and other products to meet consumer preference. Substantial research achievement toward these objectives is essential so that we may continue to eat the meat we want, at a price we can afford to pay, with profit to the livestock industry. We have 160 million people to feed now. We may have 200 million to feed 20 years from now. We must prepare to feed them.

During the 20 years past, our meat supply has more than kept pace with our population. We will eat 15 percent more meat per person this year than we ate during the 1935-39 period. Part of our increase may be attributed to the application of research results. We have improved efficiency of production in terms of yield per animal breeding unit. In these terms, efficiency of poultry meat and egg production has been improved remarkably; swine and dairy cattle have improved substantially. Nutrition and genetic research results have contributed to this improvement.

Efficiency must also be measured in economic terms. U.S. Department of Agriculture estimates of efficiency of meat production enterprises give us no grounds for complacency. Production per unit input of all factors used in production was expressed in terms of constant dollar values, assigning the 1930-33 period a value of 100. For intermountain sheep ranches, the 1949-52 value was 96; for intermountain cattle ranches 93; for Corn Belt hog-beef raising farms 137; for northern plains cattle ranches 90. We have been replacing horses with cattle during this period; not improving production efficiency of cattle breeding operations. In the 1930-33 period, we had about 72.5 million roughage consuming animal units; in the 1949-52 period, about 70 million. We haven't many more horses to replace; we will bring some new land into production; we will increase forage production on land now in use; we must also increase output per breeding unit, per feed unit and per unit of labor and machines.

Our major problem with respect to product quality is the problem of fats. This is a complex problem. From the consumption standpoint, the average person used 44.7 pounds of edible fat in the 1935-39 period which may be compared with an estimated 44 pounds this year. However, the origin of this food fat has changed sharply. Butter consumption in the 1935-39 period was almost 17 pounds contrasted to about half that much this year. Lard consumption is estimated at about 11 pounds for each of the periods.

There is an apparent though ill-defined consumer resistance to fat meat cuts. Pork loins are trimmed till the red shows. Research in the laboratories of the American Meat Institute and in other laboratories has led to rapid inclusion of stabilized animal fats in animal feeds, supplying a sorely needed

outlet for inedible animal fats.

Historically, and presently, there is a substantial relation between fatness and market price of slaughter animals. In the case of pigs, we can make these generalizations—a little later we will qualify them. The faster growing pigs tend to be fatter than the slower growing ones of the same genetic and nutritional history among pigs of the same age and sex grown at the same time and place. Fatter pigs generally have a higher carcass yield at slaughter than leaner pigs. The lean of the shoulders, hams and loins of fat pigs is generally organoleptically equal to the lean from lean pigs. I will show you later that under appropriate conditions of breeding, feeding and management that meat type hogs can grow as fast, as efficiently, give equal carcass yield as overfat hogs and higher yield of preferred cuts of superior quality.

In cattle, certainly up to a carcass fat of 32 percent, the organoleptic quality of the lean of rib and loin is improved. Below a level of about 24 percent, quality of lean in rib and loin become markedly inferior. In general, only mature cattle exceed the 24 percent fat level on forage alone. Even at this level of fatness, a substantial amount of fat is trimmed, or rejected by the consumer. There is increasing evidence that housewives prefer lean cuts of beef. Can the breeders develop cattle which produce more flesh, less external fat, with sufficient intramuscular fat to meet organoleptic standards and produce such cattle on forage and at an age and weight that will yield cuts not of excessive size?

If we must transfer a substantial portion of the 120 million additional acres which will be required to grow food crops for our 40 million additional people in 1975 from acres presently growing feed crops; if we are to produce beef sufficient to meet consumer demands, we must increase beef production from forage. We must improve efficiency through increasing output per breeding unit and per forage feed unit.

Here are some of the problems we face and a little of the research we are doing in this area. First, the agronomists and plant breeders, the soils and fertilizer and water use researchers will give the means to increase quality and quantity of forage produced. Nevertheless, we must accept the fact that both quality and quantity will be subject to species, seasonal, location and animal variation of a high order. We must learn to maximize efficiency of utilization of forage of all qualities.

Forage plants vary seasonally in protein content, vary with species as well, from 25 percent or more to 2 percent or less. Phosphorous content and vitamin A content generally vary with protein. The problem is aggravated in grasses by the fact that as season advances, lignin content increases and availability of protein is reduced. Range cured-on-the-stem grasses, straws, clovers and much of our harvested forage are deficient in protein. Feeding cottonseed cake on range helps both protein and phosphorous deficiency and may increase appetite and digestibility, too. Research at Beltsville, in Texas and elsewhere has shown the value of feeding about a pound per head per day of best quality alfalfa to meet vitamin A requirements during long periods when only dry nongrowing forage of low content is available. Supplemental phosphorus feeding as bonemeal, as soluble phosphate in drinking water or on cottonseed cake may make the difference between a 50 percent and an 80 percent calf crop.

About a year and a half ago we started a long term, 3 generation experiment on the effect of protein level on calf crop with beef cows at Beltsville. These cows are stabled as are their progeny. The work so far indicates about a 60 percent calf crop for the cows receiving about 60 percent of their theoretical protein requirement, a 90 percent crop for those receiving full theoretical requirement.

This experiment will shed light on areas of continuous deficiency when forage is generally of poor quality. This condition prevails in much of the Coastal Plain and flat woods country of the Southeastern U.S.A. Here, during the growing season, grass growth may be lush but water content is high. A thousand-pound cow needs 10 pounds of total digestible nutrients a day for her maintenance—with some grasses during high rainfall periods she would have to eat over a hundred pounds of grass a day to keep going. A lot of cows' appetites aren't equal to that and they lose flesh though they be belly deep in grass.

Then as the season advances and dry matter content of the grass increases, lignin content goes up, protein content and availability go down, and frank protein deficiency sets in. This is no simple problem. On Georgia flatwoods native range, in cooperation with the Georgia Coastal Plain Station and the Forest Service, we winter fed cottonseed cake for several years. Calf crop from the cake-fed cows was no more than five percent greater than that from the controls which averaged about 55 percent. Of course, cows held their own weight better and weaned heavier calves.

In Florida and in Louisiana it looks like we are making some improvement in calf crop with regular pasture fertilization, better pasture species and legumes—Alyce clover and indigo in Florida and Dutch white in Louisiana. One group of Brangus cows at Brooksville, Florida, each of which raised a calf last year, dropped 15 good calves this spring. General previous experience has been that cows calved only in alternate years there.

On the other hand, experiments covering several years at Ft. Reno, Oklahoma, in cooperation with the Oklahoma Agricultural Experiment Station showed that very modest winter feeding of cake—about a pound per cow per day—was sufficient to maintain a 90 percent calf crop year after year in cows kept on native range the year 'round.

Our greatest feasible gain in output per breeding unit and per forage feed unit lies in assured high percentage calf crop every year. The dry cow eats more than 3 tons of dry matter in a year's time with no return.

Our second most feasible route is the development of economical methods of carrying more of our calves over winter in the breeding areas. In the Southeast, most calves are slaughtered at weaning. The weight these calves represent is 60 percent more expensive than equal weight added by yearlings on grass. This fact is true generally, not just in the Southeast. Calf gains in the first year, since a year's cow feed must be included in the cost of these gains, cost 60 percent more forage than steer gains in cooperative research at the Woodward, Oklahoma, station, too. Of course, some folks with good, long season pasture, especially good fall pasture, will achieve efficient production of 600-800-pound slaughter calves at 10 months of age.

But since many producers must now sell weaners for slaughter or feed lot, the question of the first winter's feed must be solved if we are to have more steers on grass in such areas. At Beltsville, we have tackled this problem with identical twin calves. Genetic identity of these pairs makes data from a single pair comparison equally reliable as data from 20 pairs of unrelated calves. Thus far we have established that limitation in calories alone to maintenance requirement may be imposed during the 6- to 12-month or 3- to 6-month age periods without detriment to the subsequent performance of the calf. Present studies include protein limitations. These studies will lead to methods by which the producer can save feed and money.

Unfortunately, even abundant forage of high nutritive value doesn't solve all our problems. In order to reach acceptable market finish, cattle must eat enough feed to approach maximum rate of gain. This is sometimes done with low quality forage. The work at the Purdue, Wooster, and Iowa stations and at Beltsville is yielding valuable results on supplements which will assure

such gains. But we don't know yet how to get maximum intake. One lot of Record-of-Performance heifer calves at Front Royal, Virginia, in cooperation with the Virginia Agricultural Experiment Station, last year consumed 45 pounds of silage a day and gained over 2 pounds a day. We have had lots of thousand-pound steers at Beltsville that wouldn't eat that much silage! Why? Last month I saw a group of Hereford steers that the Louisiana folks are feeding at the Jeanerette station on a diet containing 60 percent of ground rice straw. Those steers were gaining 2 pounds a day. We'll learn.

With high quality forage, especially legumes, we have the bloat problem. Cooperative research at the Western Utilization Research Branch and at Beltsville has revealed a new lead on bloat. Apparently bloat occurs subject to three variables. When a bloating crop is grown on bloating land and eaten by bloating cattle, then bloat occurs. One factor seems to be, at least in alfalfa, the amount of saponin. At Beltsville, we have never had a fatal case of pasture bloat, even on lush Ladino or alfalfa. But cattle or sheep on such pastures at Beltsville will bloat if given press juice from the same alfalfa or Ladino in addition to the forage. They will also bloat if given a dose of alfalfa saponin.

Saponins constitute the chick growth retarding factor in alfalfa. Apparently the saponin content of alfalfa may vary with cutting and season; it may also vary with variety and location or soil. Saponin is certainly not the only factor in bloat but it may be an important one. Some day we'll be able to use legume pastures safely and produce sufficiently fat cattle without grain.

Another major factor determining output per breeding unit per feed unit and fleshing and fattening is genetic capacity.

Rate of gain of performance test bull calves on the same feed at the same time and place varies from about a pound a day to, exceptionally, four pounds a day. Much of this variation in capacity for gain is inherited. Fast-gaining bulls generally sire calves that gain faster than the progeny of slow-gaining bulls. Among bulls of the same weight, there is a very high correlation between daily gain and efficiency of food conversion. Cooperative research among 38 States, Hawaii and the U.S. Department of Agriculture has as one of its objectives the identification of lines and combination of beef cattle superior in rate of gain.

Let us consider some of the problems and research in swine production. We raise a little over 6.5 pigs per litter in this country. Some North European countries manage to raise about 8. About 15 percent of females exposed to the boar fail to settle. The average pig marketed yields about enough pork for 2 people, enough lard for three. Consumers are increasingly conscious of the waste fat on hams, excess fat in bacon.

The problem of fecundity, including failure to settle, has thus far not yielded to selective breeding. High protein levels, B vitamin supplements, adequate vitamin A are useful in successful gestation and lactation. Antibiotics have helped substantially in improving growth and viability of young pigs in the absence of specific disease.

But, general experience has indicated that fast gaining pigs tend to be fatter than slow gaining; fatty pigs generally have higher carcass yield than leaner pigs. Can meat type pigs be produced as economically as overfat pigs? Without challenging the general experience stated, I wish to present a few data indicating that selected lines and combinations of meat type pigs do gain as fast, yield as well and give high percentage of preferred cuts without excess fat. Within wide limits we can produce flesh instead of fat.

A summary of data gathered at Ft. Reno, Oklahoma, in cooperative swine breeding studies over a 3-year period compared 176 choice experimental hogs slaughtered with 110 that were overfat. The two lots were equal in dressing percentage, the choice hogs yielded 37.4 percent lean cuts as compared to 35.5 percent from the overfat, average daily gain was nearly identical and amount

of feed per hundred pounds gain was 356 pounds for the choice hogs and 359 pounds for the overfat. Another point I wish to emphasize is that meat type hogs are present in our American pure breeds. All of you know that we have used Danish Landrace and lines derived from Danish Landrace crosses with American pure breeds in our breeding studies at Beltsville. Among the 1953 spring farrowed pigs reared on performance test and slaughtered at about 210 pounds weight, purebred Berkshire pigs had 39.5 mm. (1.56 in.) of back fat and 51.2 percent yield of the 5 preferred cuts, purebred Hampshires had 39.6 mm. (1.56 in.) back fat and 50.0 percent preferred cuts, purebred Poland China had 40.4 mm. (1.59 in.) back fat and 50.6 percent preferred cuts; our purebred Landrace line had 34.7 mm. (1.37 in.) back fat and 51.0 percent preferred cuts, and our Landrace-Poland China (Beltsville No. 1) line had 40.8 mm. (1.61 in.) back fat and 50.8 percent preferred cuts. All of these hogs were clearly meat type. They were achieved with good rates of gain and good feed efficiency. Reseach in the Regional Swine Breeding Laboratory in cooperation with the North Central States has demonstrated that systematic crossing of selected purebred lines can produce an extra pig raised per litter, as much as an extra quarter pound per day in rate of gain, maximum feed efficiency and the best of these combinations under appropriate, practical feeding conditions will yield meat type carcasses at preferred slaughter weights. We have a lot of research yet to do and our industry, marketing and extension colleagues have a big job to accomplish before our average market pig produces pork for two and just enough lard for two and does it with maximum production economy. Our research results have demonstrated that the job can be done.

Now I will show you a few slides illustrative of our research program, especially in the area of carcass evaluation in sheep, cattle and swine.

In a study of yield of preferred cuts, fatness and dressing percentage in hogs, 41 selected animals representing the 7 Beltsville inbred strains, crosses between strains and American breeds were used (1). Selections of these animals were made on the live animal basis and wide variation in fatness, muscular development, type and estimated dressing yield were the objectives. The empty body weight of this group of hogs varied from 193 to 215 pounds, dressing percentage from 79.7 to 85.5 average back fat thickness of 31.0 to 54.2 mm. (1.22 to 2.13 in.) and yield of preferred cuts from 47.3 to 54.2 percent.

Figure 1 is a scatter diagram showing the regression line for the relationship between the yield of preferred cuts and dressing percentage when both are based on empty-body weight. It was evident that increased yield of preferred cuts contributed to increased dressing yield. Likewise, as shown in figure 2, a scatter showing the regression line for the relationship between the yield of fat cuts (back fat, leaf fat, plate and fat trimmings) and dressing percentage when both are based on empty-body weight, increased yield of fat cuts was associated with dressing yield.

Statistical analysis of these data showed that yield of preferred cuts contributed more to dressing yield than did yield of the fat cuts. Therefore, it is possible through breeding and selection to reduce the fatness of hogs without sacrificing dressing yield.

Figure 3 shows the relation between the percentage of separable fat in the 9 rib lamb rack and the yield of the 4 most preferred cuts based on shorn slaughter weight. The estimating curve calculated from moving averages shows that yield of the preferred cuts increased consistently with increased yield of separable fat in the rib until the latter has reached a level of approximately 31.5 percent. It has been reported by Hankins and Foster (2). that a lamb rib with 31.5 percent separable fat represents a dressed lamb carcass that is top Choice to low Prime in grade under the present grading standards for lambs. These data are from 151 lambs that represented purebred Southdown, Shropshire, Hampshire, Merino and crossbred rams, weighing approximately 75 pounds final weight.

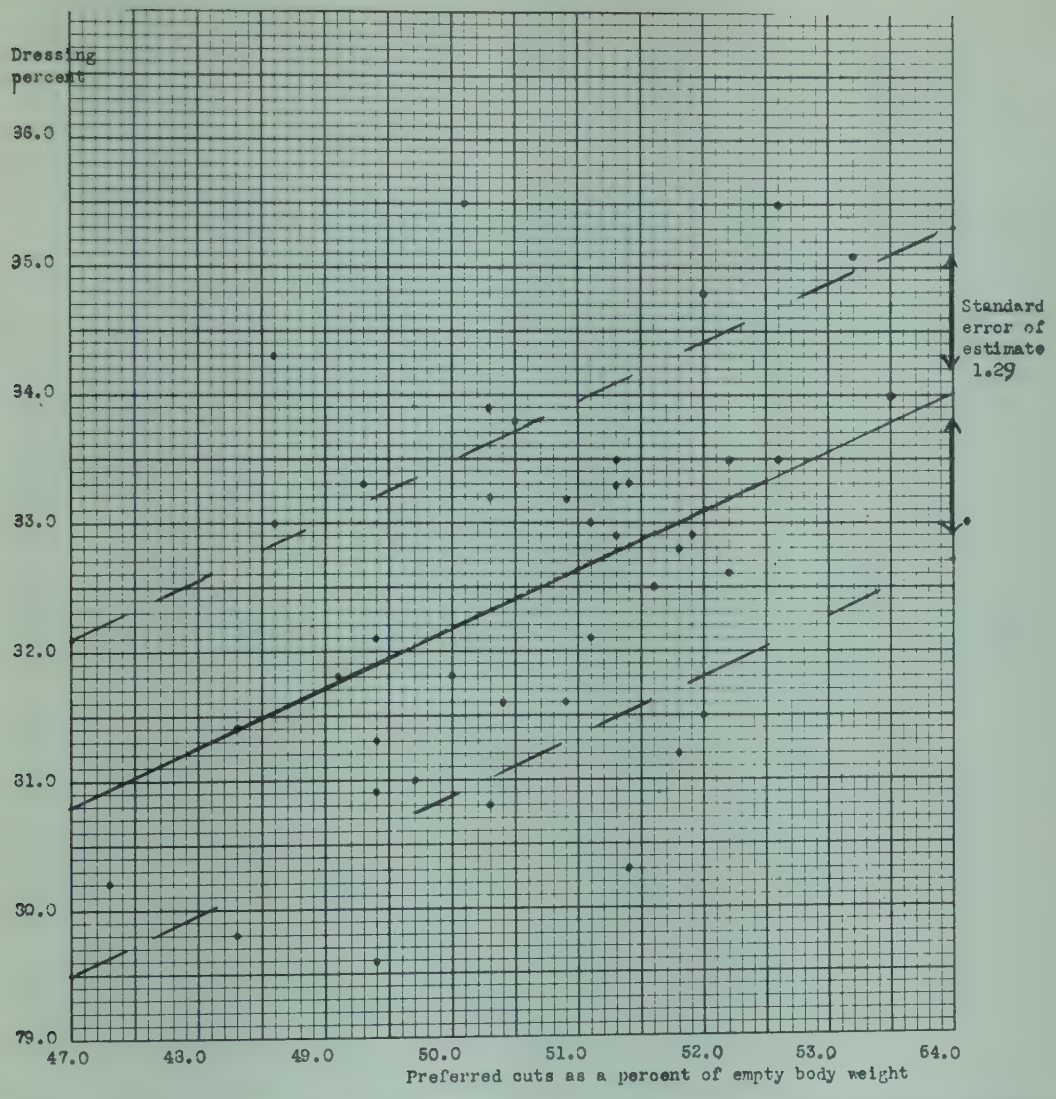


Figure 1 - Relation between the percentage of preferred cuts and dressing percentage among 41 selected hogs.

The relationship between the percentage of separable fat in the 9-10-11 beef rib and the yield of the 4 most preferred cuts based on slaughter weight are shown in figure 4. The estimating curve calculated from the moving average shows that yield of preferred cuts increased consistently with increased yield of separable fat in the rib until the latter has reached a level of approximately 31 percent. A rib cut with 31 percent separable fat represents a top Choice to low Prime grade beef carcass, according to present grading standards, as shown by Hankins and Foster (3). These data represent 317 beef and dual-purpose type Shorthorn steers slaughtered at a final feed lot weight of approximately 900 pounds.

It is of interest to note the similarity of the separable fat of the rib cuts and yield of preferred cuts representing lamb and beef as shown in figures 3 and 4. These data show that increase in yield of separable fat in the rib cut and increased yield of preferred cuts based on slaughter weight are closely associated until the fatness level in the rib sample (a good index to total carcass fat) has reached approximately 31 percent in young animals. After this period of growth and fattening has been reached it would appear that additional fat has little relation to increased yield of preferred cuts. From the standpoint of economics this would be the degree of fatness for which the producer

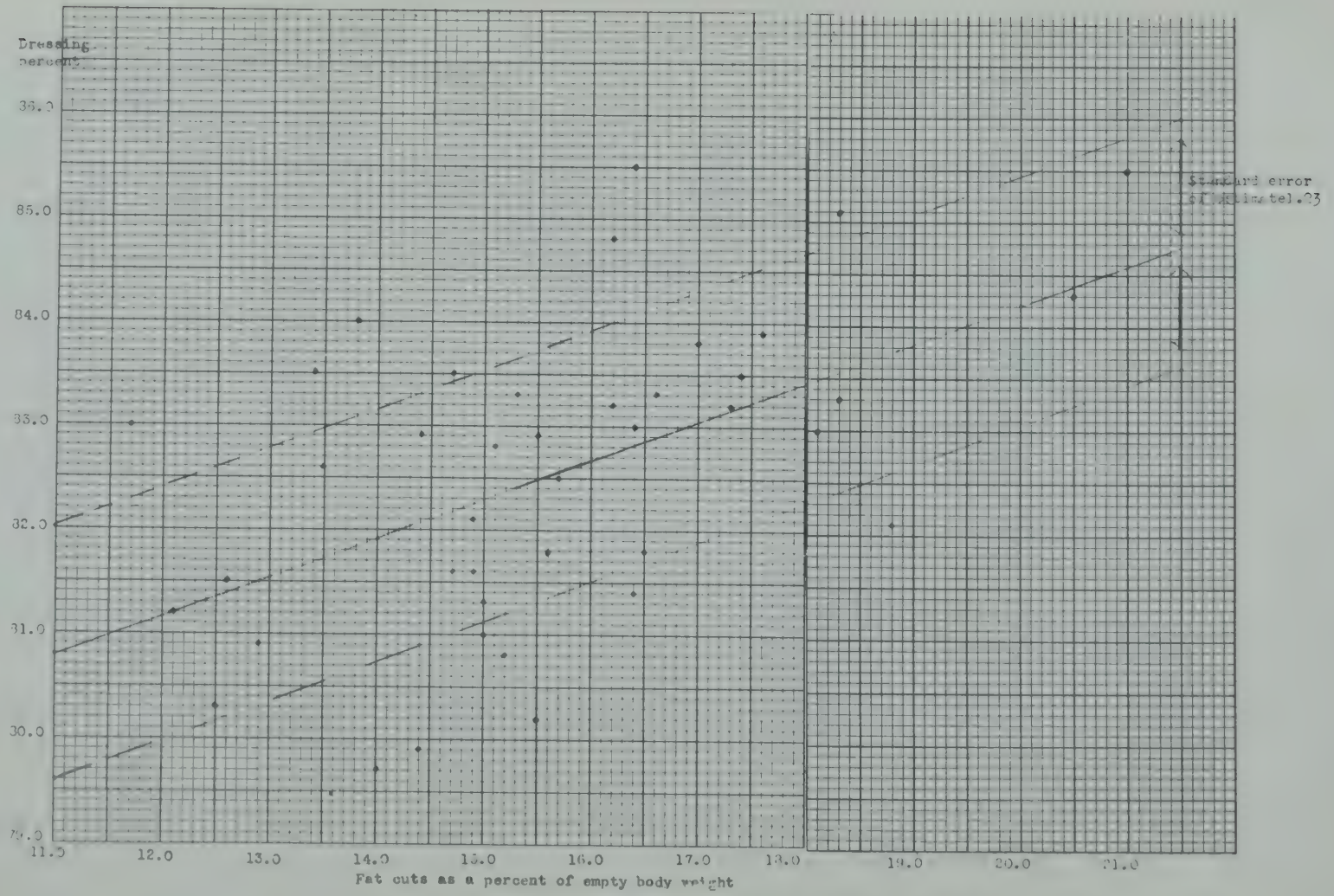


**Figure 2.- Relation between the percentage of fat cuts and dressing percentage among 41 selected hogs.**

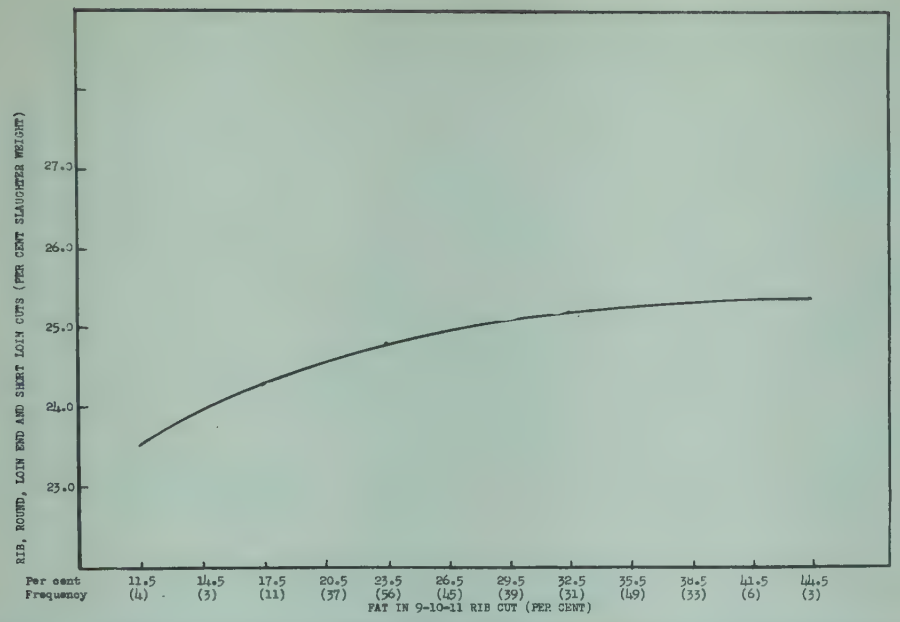


Figure 3 - Dual-purpose and Beef Type Cattle - Relation Between Separable Fats in 9-11 Rib and Yield of Rib, Round, Loin End and Short Loin Cuts.

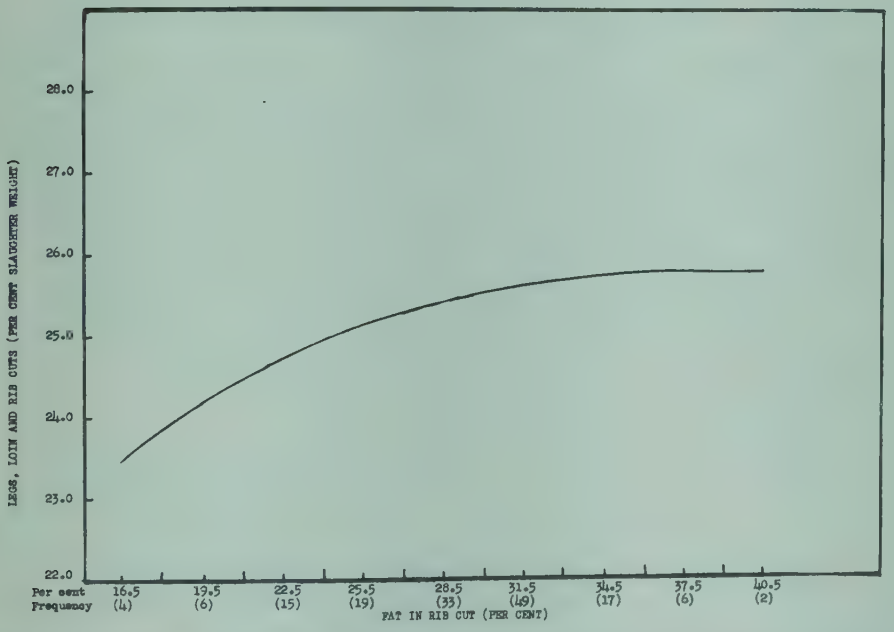


Figure 4 - Lambs - Relation Between Separable Fat Content of Rib Cut and Yield of Legs, Loin and Rib Cuts.

should strive. With respect to quality of meat, Prime grade represents subjectively the ideal with respect to proportions of lean to fat, primal cuts, fat covering and consumer acceptance.

Figure 5 is a scatter diagram showing the line of moving averages for the relationship between the percentage of separable fat in the 9- 10-11 rib cut and carcass grades in 900-pound, beef-type Shorthorn steers. Also shown is a similar scatter diagram and line of moving averages showing

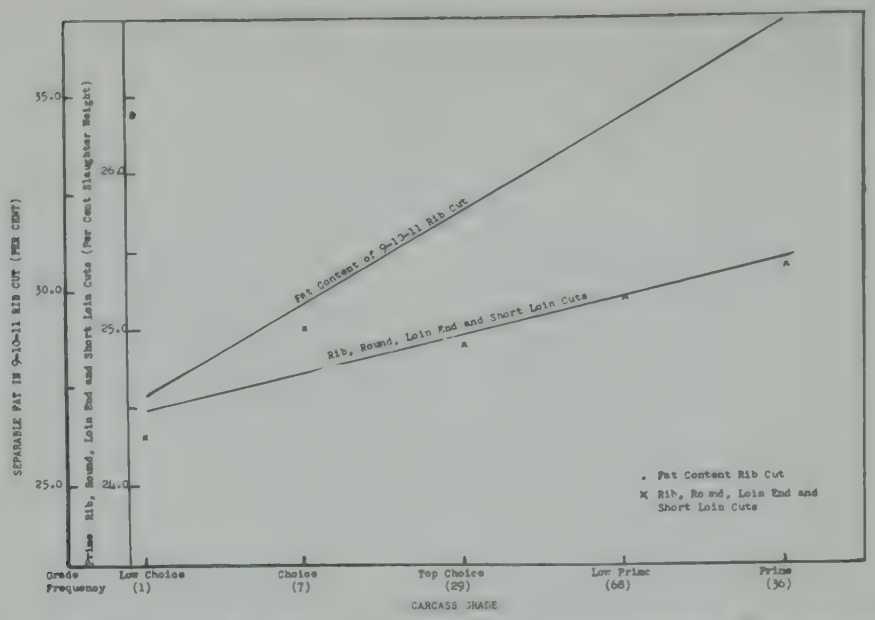


Figure 5 - Beef Type Shorthorn Steers - Relation between Carcass and Separable Fat in 9-10-11 Rib Cut and between Grade and Yield of Prime Rib, Round, Loin End and Short Loin Cuts Based on Slaughter Weight.

the relation between yield of the four preferred cuts and carcass grade from these same animals. Attention is called to the increasing differences found between yield and fatness with each increase in carcass grade. As shown previously (figure 4) after a separable fat field of 31 percent was reached, yield of cuts did not increase in proportion to the fatness increase. In the case of the beef-type Shorthorns this point was reached when the carcass was graded between average and top Choice. However, as shown in figure 5, yield of preferred cuts continued to increase. The first question that naturally arises is the possibility of obtaining fatness in the beef type animal at a given level, and increasing the yield of its preferred cuts to the higher level of 25.5 percent and above. A second question that can be rightfully asked is—with each increase of, for example, one percent in separable fat in the rib, what proportion of the increase in yield of preferred cuts can be attributed to increase in fat per se and how much to muscling?

Figure 6 is a similar diagram showing the same relationships between percentage of separable fat in the 9-10-11 rib and grade and yield of preferred cuts and grade in dual-purpose type Shorthorn steers when slaughtered at 900 pounds final feed-lot weight. Again it will be noted that increase in percentage of separable fat was more rapid than the increase in yield of preferred cuts. It is of interest to note that steers of low Utility carcass grade have approximately the same proportion of separable fat in the rib sample and proportion of the four preferred cuts based on slaughter weight. Due to type of animal it was not possible to obtain top Choice steers at 900 pounds final feed lot weight, but the indications are that if one did the yield of separable fat would be quite similar to that found in the beef-type steers. However, at the lower Choice carcass grades it required considerably more fat for a dual-purpose steer to be of comparable grade. This was due to conformation and thickness of the dressed carcasses. The same questions that were set forth for beef-type steers could and should be logically asked about this type of steer. Effort is now being directed toward the obtaining of information on these questions through selection of breeding stock and nutritional studies in which maximum roughage and minimum concentrates are used to obtain the desired finish on the test animals.

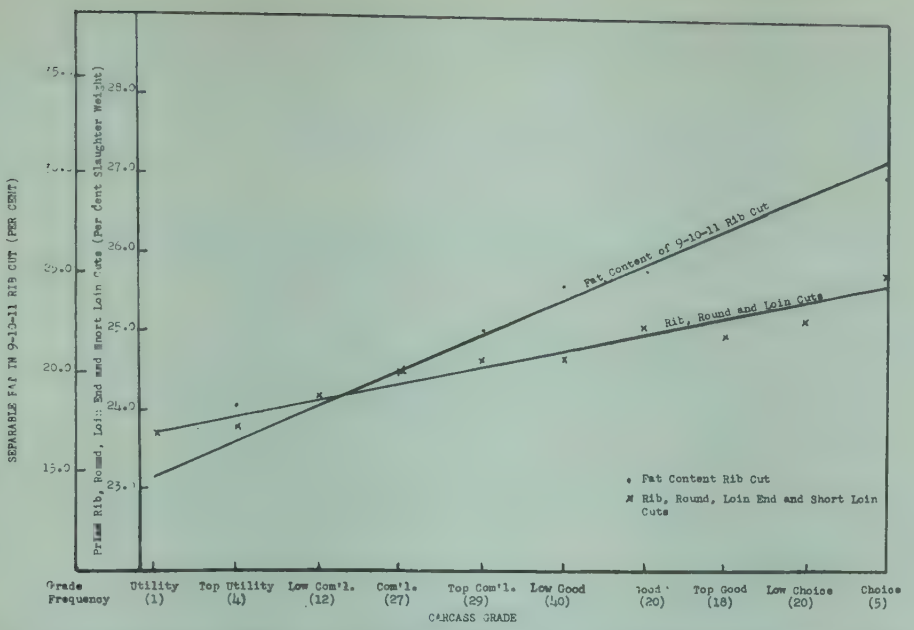


Figure 6 - Dual-purpose Type Shorthorn Steers - Relation between Carcass Grade and Separable Fat in 9-10-11 Rib Cut and between Grade end Yield of Prime Rib, Round, Loin End and Short Loin Cuts Based on Slaughter Weight.

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XII

# SOME RECENT RESEARCH WHICH CONCERNS THE ECONOMIC ASPECT OF HIDES

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With a degree of timidity I venture into the field of economics; but I shall confine it to the simple proposition that unless an object sells for more than it costs it is uneconomic.

This would require a sound method of determining the cost of the raw material, labor, and the merchandising costs of the manufactured product. The tanner is in a peculiar position. He buys a byproduct of the meat industry and sells the manufactured product, leather, to the shoe industry to be further fabricated.

Hides and skins price fluctuations formed the main interest of tanners previous to the adoption of "LIFO"** by the majority of the leather industry during the decade 1940-1950. "LIFO" made the tanner conscious that he must get the replacement value of the hides used to make the leather he is selling that day. This is a matter of good bookkeeping.

The fundamental knowledge of what hides or skins cost is in general rather vague. Of course we see the market quotation listed each day, but when we analyze the actual cost from these listings we see opportunity for great variation in value received.

Hides are most generally purchased in the green salted cured condition after being in salt for as short a period as two weeks to several years. Hides are purchased against sight drafts and thus paid for before they arrive at the tannery. And while standards have been written, still the tanner is often buying a pig in a poke.

Let us consider briefly and generally a 50 lb. hide. This hide came from a meat animal and at the time of slaughter weighed nearly 70 lbs. But in the course of curing (the application of salt crystals or immersion in a brine bath) the hide shrunk; it lost water and absorbed salt. An analysis of the cured hide would be about like this:

| | |
|---|---|
| Moisture | 30 to 50 per cent |
| Salt | 15 to 30 per cent |
| Proteins | 35 to 40 per cent |
| Fat | 0 to 10 per cent |
| Hair and adhering tiusse flesh | 1 to 8 per cent |

The great variation in the percentages of constituents is due to the natural variation in hides but a lot has to do with the man-made variables. Because only the proteins and more especially the collagen content of a hide makes leather, we begin with a working product of 1/3 to 1/4 of what we buy. It is true the cured hide yields a secondary byproduct value from hair and flesh.

** "LIFO" - Last in, first out cost system of inventory.

Since the yield of leather is measured in pounds or in square feet, it does not require a skilled economist to appreciate that water, salt, fat, or flesh do not make either pounds or square feet of leather. Hide substance and only hide substance makes leather. Kjeldahl N of hides is the equivalent of B.T.U. of coal. No sound business man would buy carloads of coal without this information.

So we buy at 20 cents a pound in the cure; this is costing the tanner 80 cents to $1.00 a pound for the leather making material.

This however is not all. The hide may have been carelessly handled in the cure cellar, storage, or shipment and be in a poor state of preservation. The hide may have a brand which involves more than half of the side. The animal may have mange, warts, grub, or any one of a dozen diseases involving lesions on the animal's skin. Many times such defects are unrecognizable until the hair has been removed before tanning. Such defects involve about 50 per cent of all hides produced. In some it involves as much as 75 per cent of the hide, while in others it involves only a minor area.

These defects add to the cost of the hide, as the resultant leather is devalued in proportion to the defect, and in the course of making leather the tanner has had to do much more work on the defective hides.

With this rather gloomy background, I should like to review some of the research of the past few years which may have a direct bearing on the economics of cattle hides.

As I have said, one of the things that would be of great economic importance to the tanner, is to know the exact amount of hide substance (that is, leather-making substance) present in the lot of hides that he buys. Of course, we realize that a lot of hides can analyze high in hide substance, and still not make good leather because of defects in the hides from brands or skin lesions. However, the tanner would be protected against paying high prices for hides which contained excessive amounts of water, salt, or filth and badly deteriorated proteins.

But let us first look at some of the difficulties in determining the amount of hide substance in a lot of hides as compared, for example, with the determination of the B.T.U.'s in a carload of coal. The coal analyst starts out with a material which, compared to hides, is quite homogeneous to begin with; he can take shovels of coal from different parts of the carload, and can make this sample as homogeneous as he desires before analysis. The total loss in value of the coal is just the weight of the sample the analyst used; it does not depreciate the value of the rest of the coal of the carload to have the sampling done.

Now, let us look at the problem confronting the analyst who wishes to determine the amount of hide substance or leather-making substance in a lot of hides. Here the starting material he has to work with is very non-homogeneous; most of the hides in the lot may be very high in hide substance, but a few hides may be very wet, or have a very high salt content, or contain a large amount of adhering flesh. Obviously if he were to use the latter hides for his analysis, he would be getting a very erroneous idea of the value of the hides in the lot. Furthermore, even if he wanted to determine the amount of hide substance in just a single hide, he would obviously get a different answer depending on what area of the hide he analyzed—whether the kidney area or the belly area, for example. If he takes samples from a number of hides in the lot, he can never reduce them to a homogeneous mixture as the coal analyst can. There is one further tremendous disadvantage in analyzing hides: the loss in value of the hide is not just the weight of the sample removed, since the taking of the sample can spoil large areas of the hides for many uses.

And so we see that the relatively simple problem of determining the actual use value of a carload of coal can turn into a very complex problem if we attempt to determine the actual use value of a lot of hides. However, we at Tanners' Council Laboratory do not believe that the problems presented here are insurmountable, and we have carried out a program of research designed to tell the analyst just how to go about analyzing a lot of hides for hide substance. This work was started by Mrs. Tancous and continued by Dr. C. Deasy and is being continued by Miss Helen Steinle.

This main problem can be broken down into two simpler problems:

(1) What area of a hide should be used for sampling; that is, what area of a hide is most representative of the whole hide?

and (2) After this area has been determined, how many hides do you have to sample from a lot in order to get the average value for the whole lot?

We have carried out a series of researches to determine the answers to these questions. In the first part of the project, experimentally brined and green salt cured steer hides were used to determine the amount of bilateral symmetry existing with respect to the hide substance, total nitrogen, moisture and ash content of a hide; that is, if you sample a hide in a given position, does it make any difference whether you take the sample from the left or right side of the hide of the animal. The results showed complete bilateral symmetry; there was no statistically significant variation between right and left sides for all the analyses. And in these experiments it was also determined that there was, as would be expected, a variation in analysis according to what position of the hide was used for sampling, and also a variation due to the hide. There was, in addition, a significant variation due to type of cure; that is, a difference between analyses of two samples from the same position in the two sides of a hide, one side of which has been brined and the other side salt cured.

While these facts would seem to be more or less obvious, it was evident that, if we were to place this project on a sound scientific basis, it would be necessary to establish these facts as a foundation for future work on the project.

We next tackled the problem as to what position of the hide should be used for sampling. We were looking for an area which, on analysis, would give us a value for hide substance which would be the average value for the hide. For this work we used packer brined steer hides.

We divided the hides into 21 areas, each 8 inch by 8 inch, and analyzed these areas to determine the amount of hide substance, total nitrogen, grease, water, and ash. We could find the average value of hide substance in the hide by averaging out over the 21 areas; the same statement would apply to the other 4 analyses. In this way we found that there was one area which was the best for all analyses; that is, in statistical language, this area gave the best correlation with the average for the side, or, in ordinary language, if you analyzed this area only you saved yourself the trouble and expense of analyzing the whole hide.

This area which is the best is the area along the backbone 20 to 28 inches from the root of the tail.

Once this had been established, it was of practical importance to determine one other fact about this area. No one wants to use an 8 by 8 inch area for analysis if a smaller one can be used. If the sampling of the hides becomes too costly, it defeats its own purpose. So it was decided that the next problem to tackle was: Does it make any difference just what particular part of this particular 8 by 8 inch area you use? In other words, are there differences within this area, or is it homogeneous? And so we determined whether it made any differences in the analyses if you used that part of the area directly over the backbone, or the part farther away from it. For this

work we used 8 by 8 inch pieces of brined steer hides taken 20 to 28 inches from the root of the tail and extending over the backbone and 4 inches to either side. We analyzed that part of the area immediately over the backbone and also the areas farthest away from the backbone on the right and left sides for our usual analyses: hide substance, total nitrogen, moisture, grease, and ash. The results that we obtained showed that there was no difference between the parts of the area. In other words, the analyses can with validity be carried out from a sampling area that is immediately over the backbone. This is a good thing, from practical considerations, for it gives a hide which is less damaged after sampling than if the sampling area were further into the interior.

The next thing to determine was point two; that is, how many hides do you have to sample from a lot in order to get the average value for the whole lot? It is obvious on the face of it that the answer to this will depend to a large extent on how greatly hides in a lot differ. If all the hides in a lot are similar, you would need to analyzed only 1 to get the average value. If the hides are all very different, you would have to analyze many. So the point that has to be determined here is, then, how great a difference exists between hides in a lot. Obviously this variation between hides will vary from lot to lot, and we are interested in getting an idea of how great this variation is. Once we have this raw data, the application of statistics can tell us how many hides to take from a lot to sample in this particular position to determine the average leather-making content of the lot. This is the line along which our research is progressing at the present time.

When this variation has been determined, then we will be in a position to begin the final series of experiments. This will involve work in cooperation with tanneries to check the correlation between our analyses for leather-making substance of lots of hides and the actual yield of leather that a tanner gets from them.

The final end result of this research may be that the tanner will be able to have a realistic standard by which to buy his raw material, and the packer will be in a position to get top price for his high grade hides.

I have given an account of this line of research in detail for two reasons: first, because I am familiar with all the aspects of this work since it is being carried on at the Tanners' Council Laboratory, and secondly, because I believe that it is an excellent example of the bearing that research may have directly on the economic aspects of hides.

I have already told you about the composition of the average hide, and you have seen that nearly 75% of the hide consists of non-leather making constituents. 75% of the shipping costs of the hide, then, is being paid for transportation of what is essentially waste material. It would benefit both packer and tanner alike if this non-leather making material could be reduced at the packers before shipping. We know that this waste material can be removed to a great extent from the hide; the trick is to remove it without changing the properties of the hide so that the tanners' job of converting the hide to leather will not be made more difficult.

Another research which promises to have definite economic bearings on hides is the work being done by Dr. DeBeukelaer at the American Meat Institute Foundation. This work has already made great progress and while it should effect the economics of hides, it must at this time be weighed for the economy of its use.

This work you know from the reports made to Industry, has proved to be effective and lots of hides so treated have been processed into both side and sole leather and when such leather is compared to the leather from similar hides not treated it was found the leather was satisfactory in every instance.

The removal of manure and other filth permits for leather which is not badly stained.

Now, the economic consideration,

First, manure and dirt have weight. The Tanners' Council in a survey found 335,000 hides to have adherent to them 800,000 pounds of manure—the freight on such lot at 75 cents per hundred (this is average) would amount to $3,900.

The hides with such manure encrustations cause badly stained leather, reducing its value as much as 25 per cent. Also such hides can not be used to make certain types of leather. When crusts of filth are present the salt is slow to penetrate and with the germ life associated with dirt and filth a much poorer cure is obtained.

Next, the cost of treatment, as well as the question of equipment must be considered as opposed to what other benefits are derived from such cleaning operations. This work continues and here is an illustration of how the economics of a process for cleaning hides must be evaluated for the total benefit versus the cost in material, equipment and labor.

Experiments to reduce the amount of moisture, salt, fleshings, and hair in order to reduce shipping costs are already under way at the American Meat Institute Foundation.

It is interesting to note the relation between the research done at the American Meat Institute Foundation and that going on at the Tanners' Council Laboratory about which I talked to you previously. Obviously it would be economically desirable if the shipping cost of hides were reduced. However, if a packer is to be reimbursed for the cost of thus preparing a lot of hides which contain only low amounts of waste material, there must be some method of evaluating the amount of leather-making substances in a lot of hides. This is exactly the problem which we at Tanners' Council Laboratory are attacking. The two apparently unrelated pieces of research done at these two laboratories are seen to dovetail exactly. The relation between the research at these two institutes is not coincidental, it is the result of the cooperation of the Meat and the Leather Industries and well thought and planned programs of research.

I do not have the time to review the many other worthwhile pieces of research which are being carried out in an effort to decrease the cost of the leather-making component of hides. For example, the so-called Dresden scalding process might well have been one of the subjects of this talk. In this process pigskins are dehaired at the packer's by scalding the animal at 60°C immediately after killing and bleeding, so that the hair comes off with the scraper without effort (1). Or I might have chosen instead to deal with the research on the use in curing of powerful bacteriostatic agents, such as sodium pentachlorophenate or sodium silicofluoride (2-5), and of the current large scale experiment on Frigorifico hides being carried out in Uruguay under the auspices of British Tanners' Ltd. and Swift's Research Association (6). And then I might have gone on to tell you of the new problem in research which the use of some of these agents introduces, such as the possible fluorine content of edible gelatin made from hides which has been processed with sodium silicofluoride (7).

There is still another most revolutionary research and development program which, while not original research in recent years, now promises to be of great economic interest. I refer to the solvent process in leather making.

As early as 1910 it became apparent that solvent tannage had many advantages, but it remained for the engineering advances of World Wars I and II to bring such practice into reality.

Today there are two solvent processes in or near to pilot scale operations. These effect economics through speed in processing time, the production of a better and more uniform product with reduced costs in materials and better handling costs.

Sole leather which today requires 60 to 90 days to complete can be made from the cured hide to finished leather in less than two weeks.

Side and light leathers have been reported capable of being tanned and finished in a matter of minutes. This process concerns the "Seco Tan" development.

Solvent process leather-making is in our opinion the process of the entire Industry in the forseeable future.

These other researches, while quite meritorious, are not new or different from what has already been done. Rather I have tried to bring to your attention some new and novel research which promises to bring economic gains to a very old industry. And though I have just skimmed the surface of this subject, I hope that I have said enough to show you how research is able to influence profoundly the economic aspects of hides.

As time will permit I should like to discuss briefly another example of the economic impact upon hides and skins. I refer to the animal diseases—collectively this is a multi-million economic loss to the leather industry and incidentally equally costly to the meat industry.

In the last decade veterinary literature contains many references to a mysterious cattle disease which because of the lack of specific cause and lesions has been referred to as X-Disease.

It is now accepted as a symptom rather than a disease—it is mainly characterized by a distinct hyperkeratosis. For many years this disease condition defied the searches in finding its cause. Only recently has it been determined that it is caused by the ingestion and absorption of chlorinated napthalenes contained in lubricating gases. It gels into the feed of cattle from the lubricant of the machines used to make pellets of dehydrated alfalfa. Then too the cattle have been affected by licking waste oil from tractors or other farm machinery. This disease reflects itself mainly on the animal skin and in its advance stage can depreciate a hide more than 50 per cent in value. In 1953 this disease has caused hundreds of thousands of dollars loss to tanners.

The research concerned was the finding of the cause and its removal—this was done in the U.S. by the Department of Agriculture personnel as well as many researchers in universities and colleges.

The purpose of all this diversified research, however, is always directed toward the same goal—making a better product at a cheaper price, a price that will give leather an even better competitive position with the newer substitutes.

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XIII

# DIATHERMAL PROCESSING OF MEAT

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Before a discussion of the application of a natural phenomenon can be intelligently conducted, an explanation of the behavior and possible cause of it should be attempted. The phenomenon we are going to consider pertains to the conversion of electrical to thermal energy. This conversion takes place in a metallic conductor, an ionic solution, and even in some so-called non-conductors. However, the mechanism of the conversion differs in each case. Thermal energy is generated in the first case by resistance to electron motion, in the second by ionic motion, and in the last by polar molecular motion. The application of the first for thermal energy generation is referred to by industry as induction heating and the last as dielectric heating. We call the combination of the ionic and molecular mechanism generating the energy diathermal processing.

To apply the diathermal phenomenon for increasing the thermal energy level of meat one should know what properties of the meat will affect this phenomenon and how much. Our qualitative and quantitative studies provided us with information adequate to satisfy our practical curiosity. Measurements were made (Table I) with different combinations of polystyrene and meat

Table I - The Capacitance of Raw Boned Ham at Room Temperature as Influenced by Condenser Arrangements

| Condenser Arrangement | Capacitance μμf |
|---|---|
| Meat condenser with inner plastic discs and "2 disc" plastic condenser in series | 4.4 |
| Ditto but without inner discs | 10.5 |
| Polystyrene condensers: Two "2 disc" condensers in series | 9.2 |
| Air condenser with inner plastic discs and "2 disc" plastic condenser in series | 3.2 |

Distance between plates 2-5/8". Plastic condenser made by inserting plastic discs between two other electrodes connected in a series or in parallel as indicated. Readings were taken with a General Radio Co. Capacity Meter.

condensers; these indicated that meat had a high conductivity as well as a capacitance which could affect the current distribution in the meat. Resistivity

as a function of temperature (Table II) was obtained; and, finally, a radio frequency bridge was developed to give more reliable dielectric constant values (Table III) as well as resistivities at different frequencies. Although this was an improvement on previous techniques of measurement, a substantiation of the linear variation of voltage with amperage had to be made

Table II - Resistance of Ground Pork with Cure as a Function of Temperature

| Temperature °C. | Resistivity OHM—Inch |
|---|---|
| -15.5 | 166 |
| - 6 | 132 |
| + 5 | 121 |
| +11 | 114 |
| +21 | 96 |
| +32 | 76 |
| +41 | 68 |
| +52 | 56 |

Table III - Resistance and Capacitance of Ground Pork at Radio Frequencies (Temp. 70° F.)

| Freq. | Capacitance μμf | Resistance OHMS | Resistivity OHM—Inch | Dielectric Constant |
|---|---|---|---|---|
| 60 cps. | - | 205 | 51.7 | - |
| 7.425 mc. | 9 | 180 | 45.3 | 150 |
| 29 mc. | 8 | 170 | 42.8 | 132 |

These readings were taken with a radio frequency bridge.

to validate the results. A modification of the sine wave form on oscilliscopic examination might indicate reactions in the meat in addition to the particle motions already described. Such a modification appeared at slightly off its peak points (Figure 1). To amplify this variation the oscilloscope was connected to show the relationship of current to voltage. Instead of the straight line expected for linear conditions a triple loop (Figure 2), single loop, and somewhat straight line were obtained for aluminum, copper, and steel, respectively. This indicated that the amount of electrode reaction was dependent upon the metal and the most stable electrode was steel. The most important conclusion of this fundamental work was that design factors for meat processing had to be defined and could not be borrowed from existing dielectric processes. In fact, this could not even be classified correctly as a dielectric process.

Armed with these fundamental values we were able to design cells which would satisfy the electrical requirements. Because of the low resistance of meat we had to make the cells long with a comparativley small diameter. For example, we used 2", 4", and 6" diameter cells, 6", 12", and 24" long. The walls had to be electrically nonconducting to prevent short circuiting

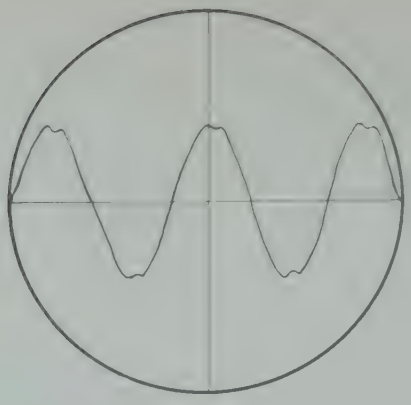

Figure 1 - Oscilloscopic wave form analysis

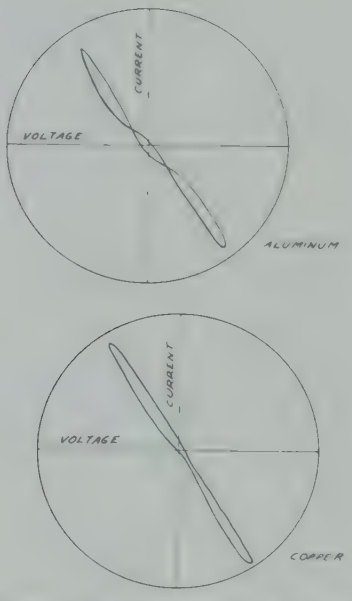


Figure 2 - Voltage, amperage relationship indicated by oscilloscope for cured, boned ham contained between aluminum and copper electrodes, respectively.

between electrodes. We used Pyrex glass tubing because of the low cost, availability, and ease of end closure using standard collars for the lipped ends (Figure 3). For the 6" diameter x 6" long cell it was necessary to use a matched circuit (Figure 4) to increase the resistance to balance the internal resistance of the generator.

The end bolted cells lent themselves fairly well to processing of cured ground pork because it was possible to provide enough longitudinal space (Figure 5) for filling in uniformly during the solid expansion that occurrs while heating the meat. In the case of cured boned hams the solid expansion was so great that this sort of simple cut did not alleviate the high pressures developed due to this solid, liquid, and gaseous expansion. To solve this problem an expansion follower plate cell (Figure 6) was developed. The springs sandwiched between the parallel plates pressed the contacting electrode against the ham during the entire processing period regardless of the meat expansion. There was enough free space between the sandwiching plates to alleviate the expansion pressure almost completely. However, it

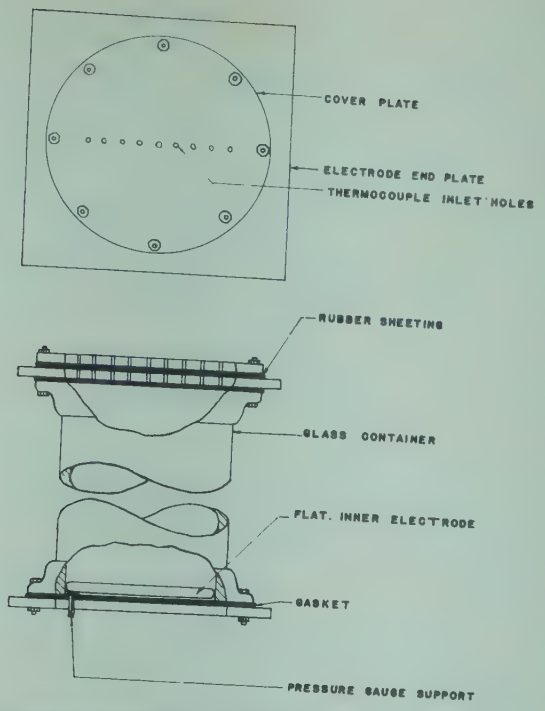


Figure 3 - Processing cell incorporating lipped ends and collars with flat electrodes for closure.

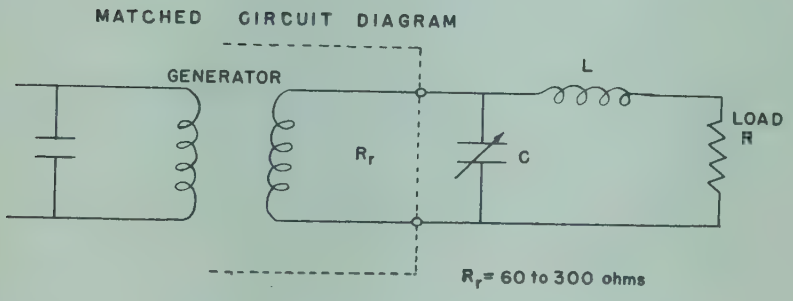


Figure 4 - Matched circuit used to satisfy the internal generator resistance.

was necessary to place a braided jumper lead between the plates and nylon spacer mountings for the springs to prevent the springs from burning up from the high current flow. This type of cell could be used for a commercial process involving an aseptic transfer mechanism because the electrode would adjust itself to the surface level variation from one ham to another as well as level change during the processing of any one ham. This transfer mechanism should be simple because no special heat transfer units would have to be built or complicated air displacement mechanisms designed.

An alternative commercial process involves the development of a plastic or insulating walled container whose caps or ends would act as electrodes after the meat was closed in the container, thus avoiding the need for aseptic transfer. The wall plastic would have to be sufficiently impermeable to gas flow to maintain a vacuum for several years and would have to be cheap,

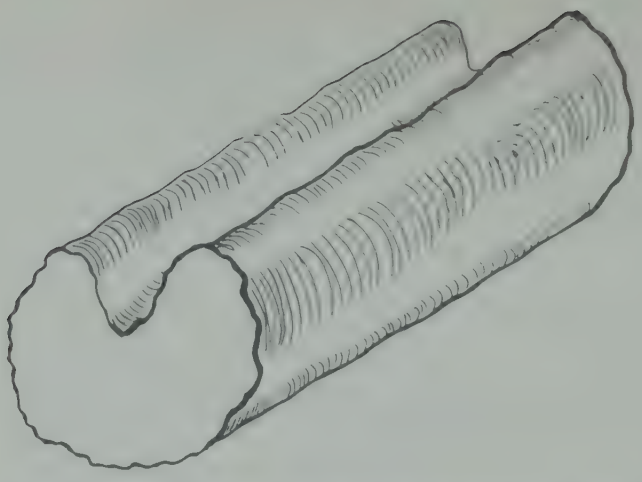

Figure 5 - Sketch showing cut in roll of meat to allow for alleviation of pressure caused by the expansion of the meat.

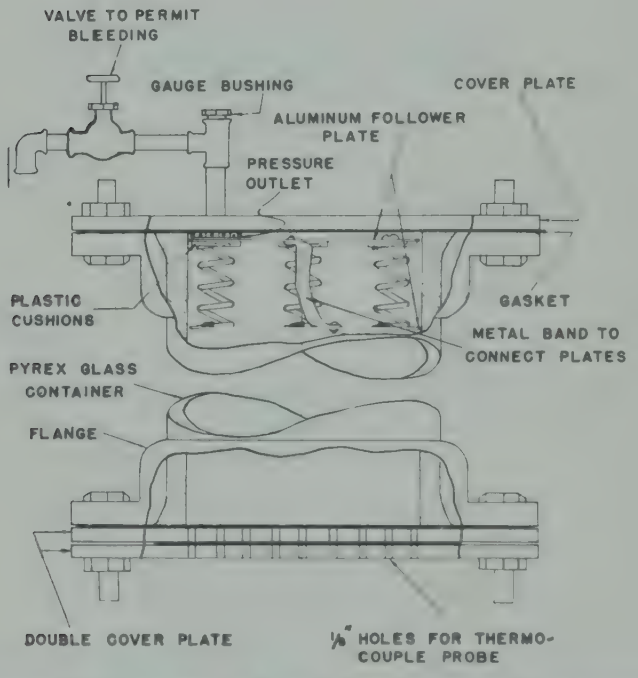


Figure 6 - Processing cell incorporating an expansion follower plate electrode.

tough, thermally stable, non-toxic, and non-odorous. Thus far a paper based. phenol-formaldehyde plastic has been tried and a fiber glass based, acrylate plastic considered. They show enough promise to justify further testing.

To simplify the closing of the plastic container, a vacuum cap closure was considered. With the cooperation of the White Cap Co. several caps were designed and tested (Figure 7). One of the first difficulties encountered pertained to the high pressures developed by the expansion of the ham during processing. We designed a concentrically corrugated cap end and found that

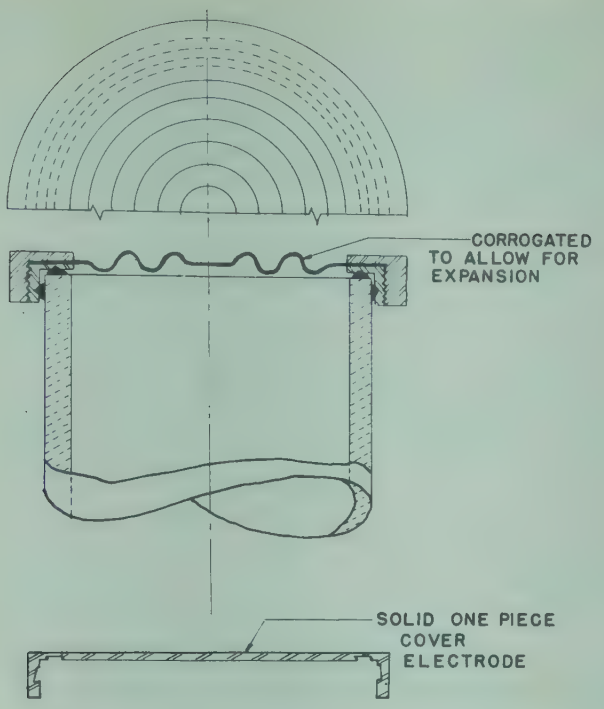


Figure 7 - Flat and corrogated caps used as electrodes and closures in the vacuum closure technique.

this was rather successful in alleviating the pressure.

To facilitate production it might be desirable to process several cells in series. If the electrical property variation from ham to ham were great enough to cause one ham to overheat while the other were to be under processed, then a series connection could not be used. We found no such indication after making three runs (Table IV) and felt the statistics of such an

Table IV - Temperature Distribution in Boned Hams Processed in Series Connected Cells at Atmospheric Conditions

| Cell No. | 1 | 2 |
|---|---|---|
| Depth Inches | Temperature* °F. | Temperature** °F. |
| 0 | 211 | 206 |
| 2 1/4 | 222 | 219 |
| 4 1/2 | 233 | 226 |
| 6 3/4 | 237 | 243 |
| 9 | 240 | 246 |
| Average | 229 | 228 |

* Average for 9 positions.
**Average for 7 positions.

occurrence would have to be determined with a large number of hams such as would be encountered in production. From the theoretical consideration there is not sufficient variation in conductivity to indicate any serious difficulty of this nature.

A possible commercial process for luncheon meat involves the continuous treatment of the meat while it is being propelled through a tube by means of a screw. The hot electrode forms the coupling between the two parts of the nylon screw and the tube acts as the ground electrode (Figure 8). The resistance of this unit was too small for the generator (15 K.W., 9 K.V., 7 mc.) and a multipass system had to be worked out (Figure 9). The current passes

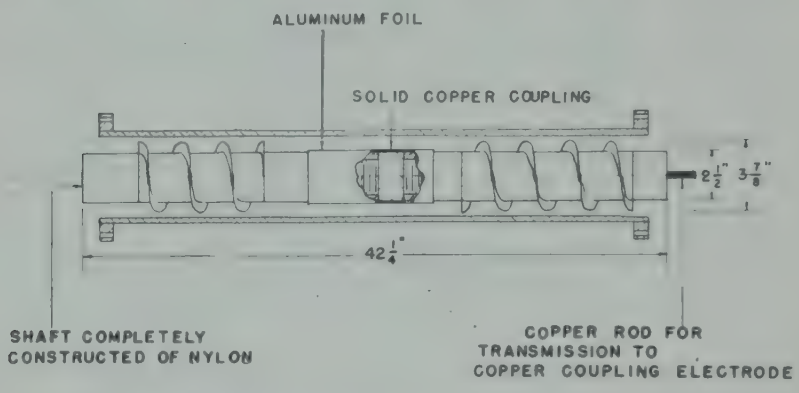


Figure 8 - Single pass current flow continuous ground luncheon meat processor incorporating nylon screw.

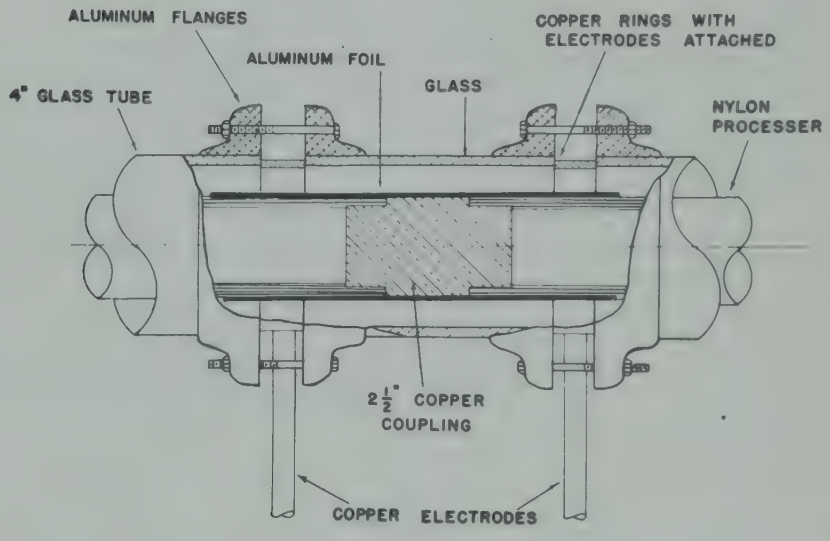


Figure 9 - Multipass current flow continuous ground luncheon meat processor incorporating nylon screw.

from the hot ring through the meat into the center electrode, along the electrode, and back through the meat to the ground ring. This system gave a very rapid heating rate.

Now that we see that the phenomenon will increase the thermal energy of cured ground pork and cured boned ham and, also, that there is a possibility of commercializing it, we should ask ourselves how this process differs from any present process so that it might be used where the present process is inadequate. Since we already stated that the process is dependent upon energy

conversion rather than thermal energy transfer, we see that a temperature differential is unnecessary. The average deviation in temperature in lines parallel to the axis of the rolled, boned, cured hams for six of our runs chosen at random was 5.3°F. However, the deviation from the average temperature of each ham was dependent upon time and rate of processing as well as the average temperature (Table V). A temperature profile along

Table V - The Influence of Sample and Processing Conditions on the Final Temperature and Temperature Variation in Processed Hams
Retort Pressure = 15 lbs.
Retort Temperature = 180°F.

| Run No. | Prossessing Time Min. | Plate Current amp. | Mean Temp. °F. | Standard Deviation °F. |
|---|---|---|---|---|
| 5 | 18 | 1.0 | 202 | 7.7 |
| 4 | 18 | 1.0 | 214 | 15.0 |
| 12 | 18 | 1.0 | 225 | 4.2 |
| 17 | 9 | 1.3 | 231 | 13.2 |
| 19 | 13 | 1.0 | 234 | 13.8 |
| 22 | 10 | 1.3 | 250 | 9.7 |

the diameter of the 4" rolls of ground cured pork indicated a bi-modal curve (Figure 10). A logical analysis of the physical situation indicated that since the meat was doing the heating the container derived its heat from the meat adjacent to the container. A simple calculation based upon the specific heats of the glass and meat and relative weights affected smoothed out the curve.

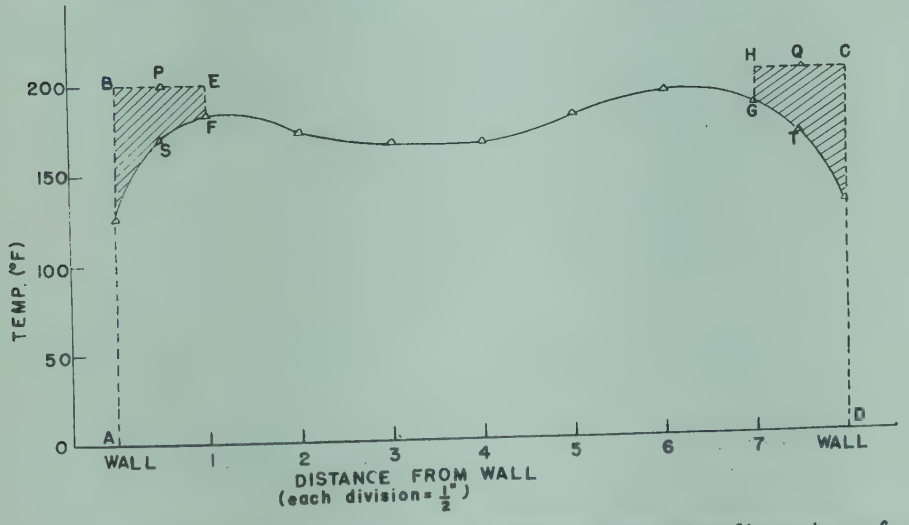


Figure 10 - Temperature distribution along the diameter of a gylinder of pork luncheon meat.

Measurements made where the container was heated by an external source, steam in this case, verified this theory by giving an essentially straight curve. These longitudinal and cross-sectional profiles substantiated the theory that meat processed diathermally might attain elevated thermal levels uniformly in spite of the physical and electrical non-homogeniety of the meat. The practical significance of this statement is that a portion of meat does not have to be over-done in order to attain a minimum sterilization level on the other portions.

Another advantage of this process over the conventional one is in the rate of processing per unit. This is a two-fold advantage. It makes possible a high production rate and, at the same time, it allows a high temperature short time process. If a 100 K.W. generator were to be used a production rate of 2,425#/hr. might be attained, or close to a ton and a quarter per hour. This is assuming that the full capacity of the generator could be utilized. Since series connections were shown to be applicable and also since an increase in packing pressure increases the amperage per cell tolerance, this assumption seems reasonable. Fortunately, an increase in amperage also increases the conversion efficiency (Table VI).

Table VI - Efficiencies Calculated with Data Collected from Bakelite Processing Cells
Controlled Conditions: 15 lbs. Retort Pressure
180°F. Retort Temperatures

| Plate Current amp | Grid Current amp | Net Current amp | Efficiency Based on Net Current (%) |
|---|---|---|---|
| 0.80 | 0.370 | 0.540 | 35.0 |
| 1.00 | 0.360 | 0.640 | 38.0 |
| 1.30 | 0.340 | 0.960 | 46.8 |
| 1.60 | 0.230 | 1.370 | 60.0 |

The short time high temperature process allows high lethality values to be attained over a short time period, thus giving F factors sufficient for sterilization without undesirable changes in flavor and texture of the meat. Mr. F. E. Satchell emphasized this latter point when he presented the paper entitled "High Frequency Dielectric Processing of Meat" at the Third Conference on Research. In fact, these factors motivated B. W. Beadle and Jay Bowman to conduct extensive experiments in the Foundation laboratories during and immediately following World War II in the possible application of dielectric processing to meat. This factor is strikingly perceptible when a comparison of the time-product temperature curves for this process and the conventional steam process is made (Figure 11). The proof of the pudding, meat that is, was in its tasting and taste panel results have usually indicated a preference for diathermally processed pork luncheon meat over the conventionally processed product. Diathermally processed "sterile" cured boned ham has been found by Foundation staff members, as well as by some industrial representatives, to be completely acceptable in flavor and texture. It cannot be compared to similar ham produced commercially, because it is not possible to produce an acceptable sterile whole boned ham by conventional processes.

In summary, our results have indicated that the application of diathermal processing to cured ground pork and cured hams allows a uniformly high thermal energy to be attained efficiently by the product in a matter of minutes. This in turn allows total sterilization with a minimum of product deterioration, to such an extent that a diathermally sterilized ham compares very favorably with one that has attained only a pasteurization level using the conventional steam process. This process would make it possible to produce a stable canned boned ham that could be transported and distributed

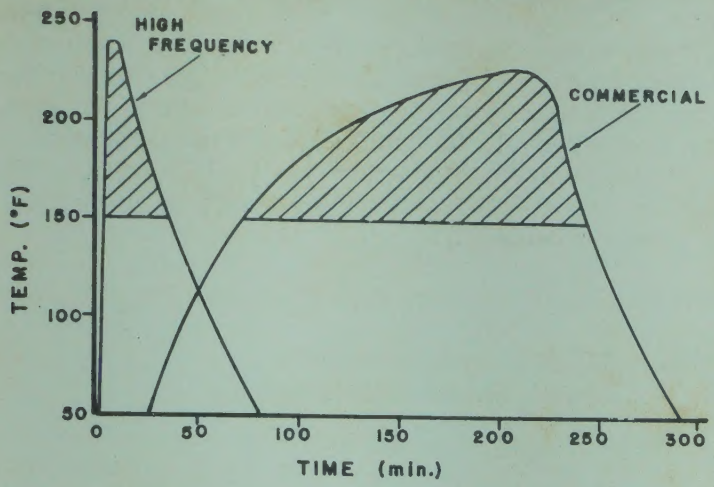


Figure 11 - Time-product temperature curves for the diathermal process and conventional steam process.

without refrigeration. Resulting savings and increased satisfaction by wholesaler, retailer, and consumer might well justify the possible additional cost of equipment and processing by the diathermal method.

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